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ACHIEVEMENTS IN BIOCHEMISTRY

- Communist China -

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## ACHIEVEMENTS IN BIOCHEMISTRY

- Communist China -

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## NEW CHINA'S ACHIEVEMENTS IN BIOCHEMISTRY

Following is a translation of an article entitled "Hsin-chung-kuo sheng-wu-hua-hsueh ti ch'eng-chiu" (English version above) by Liang Chih-ch'uan, Shen T'ung, Ting Yen-chieh, Liu Pei-nan, Li Shih-o and Wang Shih-chung in Ch'ing-shan Chien-kuo Shih-chou-nien I-hsueh K'ue-hueh Ch'eng-chiu Liu-Pen-chi (Collected Essays on the Achievements of Medical Science in Celebration of the Tenth Founding Anniversary of the Nation), Vol 1, compiled by the Ministry of Public Health, Peiping, 1959, pp 125-130.

Since the liberation of the whole country in 1949, research work in the field of biochemistry has achieved great progress because of the close attention and support of the Chinese Communist Party. Research organizations in biochemistry include the Academia Sinica, the Institute of Chinese Medical Science, Institute of Agriculture, biochemistry research and teaching groups of various medical colleges and the research units of different productive enterprises. Laboratory equipment such as electro-microscope, electrophoreses, low temperature centrifuge, ultra-centrifuge and other modern instruments have been increasing day by day. At the meantime, we are able to make various modern instruments and biochemical agents, and many new products have been discovered. In terms of reference materials, new editions of "Biochemical Terminology", "Biochemical Digest", "Selected Translations of Biochemical Articles", and translations of biochemical textbooks and references have been published. Various medical journals and medical reports about nutrition were also published. All above sufficiently explained that the science of biochemistry has been liberated from the semi-feudal and semi-colonial thinking after the liberation of the whole country, and has started to develop actively in our country.

Since liberation, the biochemical research workers have done certain studies in the fields of protein, enzyme, metabolism, traditional Chinese medicine and pharmacology.



clinical biochemistry, micro-organism, biochemistry of parasites, vitamins, hormones, and nutrition. Among these, the achievement in protein and enzymes researches are the most impressive. Studies in clinical biochemistry are very popular, and the results have been used widely, but they still need to be raised to a higher level. Studies of traditional Chinese medicine and pharmacology have also had an excellent beginning. Following is a summary of the achievements in the field of biochemistry.

### I. Protein

Protein research has received broad attention both from practical and theoretical point of view. Research work in plasma protein and tissue protein deserves special mention here; the former has a practical significance and the latter has a theoretical significance.

In the field of plasma protein study, some research units have gained considerable achievements in the preservation of liquid plasma, the production of non-specific plasma and the production of dry plasma, but preparation of non-specific plasma still has some problems which need to be solved before being broadly used clinically.

The experimental medical research unit of the Institute of Chinese Medicine has studied the relationship of antigenicity and degeneration by using pure plasma protein. They studied the effects of radiation to the physical properties and antigenicities of bovine serum albumin. They also started the study of fractionation of human plasma protein.

Besides, some research units have prepared the oxy-polymer gelatin alba, and also pointed out the shortcomings when it is used as a plasma expander. After years of research, the preparation of protein hydrolysate used as a parenteral nutritional solution has obtained a great success.

On muscle protein, the biochemical research units of the Academia Sinica has done a series of systematic research on myoglobin, including the method of preparation, the determination of the physico-chemical properties, and the analysis of the amino acid group. Myoglobin is a new kind of muscle protein discovered in 1946, and the properties of such a protein have been interpreted in detail.

Some work has been started on the brain protein and tumor tissue protein. Regarding brain protein research, they studied the isolation and purification of nucleo-protein or lipo-nucleo protein, and its composition. Meanwhile also under study is the isolation of osseous albumin and yerve albumin. The studies of tumor tissue protein include the isolation of Rence-Jones protein, the analysis of asine acid and its antigenicity. Recently, the Experimental Medical Research Department of the Chinese Medical Science Institute has done some primary research on the antigenicity of Ehrlich ascites carcinoma. It has also studied the isolation of antibody and its reaction in animals.

As far as protein research methods are concerned, some work has been done such as the application of paper electrophoresis and the improvements of paper chromatography and osmometer.

## II. Enzyme

The research of enzyme has obtained a great success because they have built up an excellent foundation in basic theoretical studies. The biochemical research unit of Academia Sinica has conducted a series of studies on cytochrome and its connection with the enzyme system, and it has proposed an excellent method on the study of the relations between the complicated enzyme groups. It has also done some research on the mechanism of the enzyme system. A new method concerning the preparation of cytochrome C of animal's myocardium and enzyme was proposed. By using the inhibitory action to the succinic dehydrogenase and the cytochrome oxidase, the function of bile salt action is explained as between cytochrome b and c. Furthermore, the unit has prepared a kind of more purified enzyme during the succinic dehydrogenase research, and it has also done some close observation on the properties of enzyme and its co-enzyme. Recently, the unit has discovered the existence of alkaline pyridine-nucleotide hydrogen-transferase, and has proved it to be different from the previously described acid hydrogen-transferase. Research has been carried out on the mechanism of xanthine dehydrogenase and the comparative study of this enzyme's hydrogen-receptor. Research on the mechanism of poly-phenol oxidase was also conducted.

In the area of enzymes connected with phosphoric acid metabolism, the members of the unit have conducted research on acid phosphatase from yeast and green lentils sprouts,

and the research includes isolation and purification of acid phosphatase and the observation of its properties and mechanism. They also did similar work on acidifying enzyme of soy bean sprout. By using the synthetic acetyl-phosphoamine group as a substrate compound in animal experiment, they studied the properties and distribution of phosphatase, and discovered that the highest activity of this enzyme is in the spleen. Besides, they also did a very thorough study concerning the characteristics of phosphoprotein and phosphatase, and the distribution of this enzyme during the embryo development stage of chicken.

In the area of enzymes connected with nucleic acid metabolism, various studies have been conducted, such as isolation, purification and the properties of de-ribodese-phospho-aldehyde, peroxidase in rats' liver, ribonuclease in pig's pancreas or the cow's pancreas. Similar researches were conducted about mannitol-isomerase, phosphorylase, and the serum transaminase.

They did some observation on the enzyme's property and its distribution in vivo under different physiological conditions such as the distribution and properties of phosphoprotein-phosphatase and transaminase during the embryo development stage of chicken. The Biology Department of Peking University started research work on the cholinesterase and other series of enzyme in the brain during the individual and systemic development stage. Besides, they also studied the changes of such enzymes during their individual development stage as amylase, ptyalin, pancreatin, pepsin, adenosine triphosphate, and the acid or alkaline phosphatase. There are also many reports concerning the enzyme activity of tumors in animals, such as the research report on alkaline and acid phosphatase related to GRCH/15 sarcoma and Rous sarcoma. The Experimental Medical Science Research Institute of Academia Sinica has done some experiments on a series of enzyme activities of Lympho-sarcoma in mice; this is only a beginning.

### III. Metabolism

Since the liberation, the research work in metabolism has undergone great progress both in quantity and quality. Especially in the last few years, research in brain metabolism as well as the metabolism of the traditional Chinese medicine and drugs has an excellent beginning.

In the area of protein metabolism, the main study was concerning protein metabolism in relation to animal nutrition, such as the effects of enzyme activity in a rat's liver while it was on a protein deficiency diet and a normal protein diet, the effects of vitamin B<sub>6</sub> on nitrogen-metabolism and also on transaminase in rats, the composition relations between riboflavin and transaminase, and also the effects of nitrogen balance to the rats on a starvation diet and those on a normal diet. All of the above are very meaningful experiments. The study of amino acid metabolism deals mainly with the intermediate metabolism of tryptophan, and its metabolic changes during Vitamin B<sub>6</sub> deficiency. Besides, they also did some study on cysteine-desulfurase of the regenerated liver.

The biology teaching and research group of the Shanghai Medical College No 1 has observed the transaminase action in the liver and brain tissue of five different kind of vertebrate animals. This study has a significant meaning in the field of comparative biochemistry.

As far as the study of metabolism of the brain is concerned, the Biological Department of The Peking University has determined the activity of transaminase, peroxidase, and the amino-acid kinase in the brain under different functional mechanism. They determined the glycogen contents in the brain. They studied the transaminase, acetylcholinesterase, 6-glucose-phospho-dehydrogenase during the stage of the individual development and systemic development. Other studies were also carried out on the analysis and observation of nitrogen-content in the brain and its transaminase changes.

Besides, our biochemical research workers have also done great deal of research about traditional Chinese medicines and drugs, such as the anti-microbial action of chang-lien, the metabolic effects of tang-kuei in mice, and the physiological action of jen-sheng in the treatment of diabetes. Also, they did some work on the metabolic effects of certain Chinese drugs such as kan-ts'ao ching-chia, huang-ch'i and ti-huang. At present, they are doing study concerning acupuncture and cauterization.

#### IV. Clinical Biochemistry

There are many biochemical diagnosis problems worth mentioning, such as the electrophoretic analysis of serum

protein and lipoprotein in normal individuals and in abnormal individuals, the determination of cholesterol and phospholipid in the blood and also their distribution in  $\alpha$  and  $\beta$  lipoprotein. As for the enzymes, research has been made about the changes of blood glutamin-oxaloacetic acid transaminase, amylase and cholinesterase in connection with various diseases. As for Hormones, they studied 17-ketosteroid, 17-keton cortical steroid cholesterol and aldehyde cortical steroid cholesterol in the urin of normal and sick persons. Besides, they also studied the ammonia content in the blood of patients who have liver or mental diseases. Above studies are very helpful in the early diagnosis and prognosis of various diseases.

Before the liberation, the biochemical research about occupational diseases is completely a blank. Since the liberation, owing to the special attention to the workers' health by the Communist Party and Government, many medical scientific workers have conducted studies about the prevention of occupational diseases. For instance, they have done some research on the early biochemical diagnosis of various occupational disease related to lead, benzene, mercury, manganese and silicon. Besides they also did some research on Ketonic acid and water electrolytes in workers who were working in high temperature work shops.

In the area of pathological metabolism, they have studied the ketone metabolism in relation to Wilson's disease, the metabolic condition of N.P.Ca, uric acid, creatine, creatinine and Vitamin O in relation to Cushing's disease, and nicotinic acid and reaboflavin in relation to stomatitis and glossitis.

A lot of research have been done on the methods of laboratory examination, such as the determination of protein, enzyme, glucose, N-P-N, urea, uric acid, ammonia, cholesterol, lipoprotein, and many methods have been improved for the determination of these substances or other inorganic electrolytes in the blood and urin. There were some reports on the use of electrophoresis and chromatography. As to the test methods in the field of biochemistry, there were many improvements. During the great leap forward, many laboratories have succeeded in one study which is very valuable and should be mentioned here, that is the microanalysis or ultra-analysis method. The advantages of this method are: a) As only a small amount of blood is needed, the patients are relieved of bad psycholo-

gical effects. b) A lot of drug is saved. c) Many instruments are saved. d) As only a small amount of sample is needed, they can do 2-3 determinations which can give more accurate reports about the samples under analysis.

While conducting the clinical diagnosis and introducing new methods, they have determined a biochemical index for the normal person. This normal index of biochemical composition in blood or in urine is a very valuable indication of the health of the people in our country.

Some achievements were made in the manufacture of biological products. Before the liberation, most of the clinical biochemical products were imported from foreign countries. They were very expensive and the supply was not always on time. Since the liberation, this long-term situation of dependence on foreign countries has gradually changed, and we could make some of the biological products ourselves. For instance, in trial making bile salt,  $\gamma$ -globulin and amine acid compounds, waste or inexpensive materials were used as raw materials in accordance with the rules of mass production. But the types of products are very few, and we still need to advance to a higher level.

#### V. Hormones

The biochemical research work on Hormones can be summarize as follows: In regard to hormones' effects on metabolism, the biochemical teaching and research group of the Sze-chuang medical college has studied ACTH's effects on liver glycogen composition in normal and Vitamin C deficient guinea-pigs, and the adrenocortical hormone's effects on the liver glycogen composition in mice. In regard to hormone effects on the tissue composition and enzymes, they discovered an increase of pancreatin Zn content in mice after the injection of bovine anterior pituitary hormone, and the disappearance of adrenal cortical phosphatase in male mice after the injection of estrogen. About hormonal secretions, the biochemical teaching and research group of the Wu-han Medical College has discovered a pituitary stimulating factor in tissue extract which can stimulate the pituitary gland to secrete ACTH, and also studied its property. As for the bioassay in hormones, there were some reports about the use of the dipropionate treated rat's uterus for the determination of posterior pituitary hormone reagents. As to the determination of hormone or its metabolic products in blood and urine, they have conducted

serum P-B-I determination and reported various steroids in the urine. Concerning the effects of preventive medicine and drugs, there were reports about the adrenal cortical hormone in connection with various kind of Chinese drugs such as jen-sheng, ching-chiu ho-shou-wu, kan-ts'ao. There were reports about the fact that thyroid hormone will decrease antimony tolerance in the treatment of schistosomiasis in animals, and reports about the preparation of iodized oil which can be used for the prevention and treatment of endemic thyroid enlargement.

## VI. Vitamins

The following are the main research items in vitamin biochemistry:

In the area of test methods, they have studied and improved the determination of carotin, Vitamin A, Vitamin D, Vitamin C, Vitamin B<sub>1</sub>, and Vitamin P. Most of these mainly concern the test method of Vitamin C.

In the area of vitamin metabolism, they studied the relation between serum complement titer and blood Vitamin A and C content changes; the influence of riboflavin deficiency to the liver serum protein of rats to the regeneration of the liver and serum protein, and to the composition of myoglobulin. They also studied the liver glycogen production and gluconeogenesis in Vitamin C deficient guinea pig.

As to the biological synthesis of vitamins, some scientists have discovered the highest amount of product is obtained when millet, bean curd refuse or gluten are used as a culture medium in the study of bacterial synthesis of riboflavin. The inositol could stimulate the respiration, growth, glucose and nitrogen utilization of synthesis riboflavin bacteria, and also could decrease its aging and autolysis action. The purin of bird feces has a promoting action on inositol in the growth of synthesis of riboflavin bacteria. In the study of biological synthesis of antiscorbutic acid, they discovered that L-glucuronic acid- $\gamma$ -lactone, or L-galacturonic acid- $\gamma$ -lactone, is the enzyme of antiscorbutic acid in the liver of rat. It is mainly distributed in the liver's mitochondria cell and this enzyme is closely related to riboflavin.

## VII. The Biochemistry of Micro-organism and Parasites

The biochemical research in the field of micro-organism and parasites in our country have been gradually developing since the liberation. Research work in micro-organism is mainly divided into two parts.

1) In the research of inhibitory action of bacteria, most of the work was done on the antimicrobial action of the antibiotics. The organic chemistry research units of the Academia Sinica has conducted studies on the antimicrobial action of chloromycetin and the effect of aureomycin to the respiration of *E. Coli*, but the effect depends upon whether there is nitrogen source in the culture medium. The pharmacological research units of the Academia Sinica has observed that terramycin could decrease the synthesis of 4-aminobutyric acid by *E. Coli* in the inorganic nitrogen culture solution, but at the same time it could increase the amount of valine. The biological teaching and research group of the Szechuan Medical College has observed the effect of kuang-lien complex to the respiration of *staphylococcus aureus*, decarboxylation and dehydrogenation of many other organic acids.

2) More work was done on the physiology of antimicrobials and fungus combination for the production of antibiotics. They not only conducted experiments for the production of penicillin, streptomycin, aureomycin, and terramycin, but also sought for the substitutes of the components in the penicillin culture solution. For the purpose of increasing aureomycin production, the Plant Physiology Research Institute of the Academia Sinica has also studied the physiology of *streptomyces aureus* and its connection with aureomycin synthesis, especially the effect of inoculated culture medium to aureomycin metabolism. They have proved that the phosphoric acid salt could inhibit the activity of 6-phospho-glucose-dehydrogenase in *streptomyces aureus*, promote the oxygenation of triose-phosphate which may prevent the metabolism of hexose-phosphate and the combination of ironion and aureomycin could influence the production amount of this antibiotics.

Besides, the Pharmacological Research Institute of the Academia Sinica has studied the K effects to the growth of *streptomyces* and the production amount of streptomycin. During the research in physiology and morphology of the



bacterial synthesis of riboflavin, they have proved that inositol could increase these bacteria's growth and increase the production amount of riboflavin. The Micro-organism Research Institute of the Academia Sinica has studied the activity of many gluten enzymes such as protease, amylase and glucogenic enzymes.

As for studies in the field of parasites biochemistry, the main research was done on the chemical composition of parasites and their metabolism. Some units have conducted research on the nitrogen, phosphorous glycogen distribution in *Schistosoma japonica*, and compared the female and male parasite's protein contents and their characteristics. They also compared the acetylcholinesterase activity in both male and female parasites.

Besides, they also studied the oxidation and reduction of enzyme and phosphatase in *Fasciolopsis buski* and *Fasciola hepatica*.

The Parasites Disease Research Institute of the Academia Sinica has discovered that the fermentation ability of *Schistosoma japonica* was closely related to the composition of the culture solution when they were studying the glucose metabolism of these parasites, that the concentration of phosphoric acid salts may possibly be the factor which decrease its respiratory quotient, that riboflavin and antiscorbutic acid may increase its respiratory quotient to 25 percent, and that cystein, Ca-pantothonate and adenosine-triphosphate also have the similar action. This explained that the increased activity of this parasite is closely related to Co-enzyme A. Vitamin K<sub>2</sub> has a very strong inhibitory action on *Schistosoma Mansoni*, but has a stimulating action on *Schistosoma japonica*. The tartar-emetic and iodo-acetic acid have the inhibitory action on this worm's fermentation. Besides, they have observed L-hydroxyproline, L-proline, L-cytine, DL-valine and DL-phenylalanine could prolong the life span of *Schistosoma japonica*.

#### VIII. Nutrition

By using paper chromatograph, they have studied the amino acid composition in traditional Chinese gelatin. The result showed that the composition is similar to that of gelatin alba. There were many improvements in the quantitative analytic method of phenylalanine and lysine in micro-

organism and also discovered that the lysine contents of gluten is closely the animal food contents.

By using the methods of rat growth and nitrogen-balance, they discovered that the steamed soybean has a higher nutritional value than the raw soybean and fried soybean. By using the rat's body weight as an index for searching the most suitable ratio between soybean and grains, they discovered that the most suitable ratio between grain protein and soybean protein was 1:2 (millet and soybean) 1:1 (sorghum and soybean), 2:1 (corn and soybean). They couldn't find a suitable ratio between rice and soybean, perhaps because correlation action between rice and soybean is not so strong. Therefore their effect was not so obvious. That Riboflavin is an essential factor for myoglobulin synthesis has been shown in the study of feeding the rats.

In preventing the oxidation of fat in dry food, they find out that lard contains little unsaturated fatty acid and natural anti-oxidant, but crude soybean oil contains more easily oxidized unsaturated fatty acid and natural anti-oxidant. So by combining lard and soybean oil, the anti-oxidation effect will increase to a much higher level. The most suitable ratio between crude soybean oil and lard is 1:5; this result has a practical value.

The above is a summary of the achievements in the field of biochemistry, especially in the field of medical biochemistry.

At present, the medical biochemistry and the whole medical science are developing toward the same direction, that is, to eliminate the major diseases and to systematize the study on traditional Chinese medicine and drugs. The important point is the study of medical science by combining east and west, native and foreign methods. At the same time, the study of basic theories should not be neglected.

## STUDIES ON METABOLISM

Following is a translation of an article entitled "Wu-chih tai-hsieh ti yen-chiu" (English version above) by Li Shih-c and Shen T'ung, in Ch'ing-chu Chien-kuo Shih-chou-nien I-hsueh K'c-hsueh Ch'eng-chiu Lun wen-chi (Collected Essays on the Achievements of Medical Science in Celebration of the Tenth Founding Anniversary of the Nation), Vol. 1, Compiled by the Ministry of Public Health, Peiping, 1959. pp 130-134.

Various substances in the living body is continuously going through all kinds of metabolic reactions. The mechanism of metabolism is very complicated and also is the most important topic in the study of biochemistry because all these reactions are related to each other. Since the liberation, there is a great progress in this field both qualitatively and quantitatively. Especially the study of brain metabolism and research in traditional Chinese medicine and drugs (reported in other articles) have an excellent beginning after the liberation. Some research work on vitamin metabolism and hormone metabolism were also done.

### 1. Amino acid metabolism

The most important research work in this field is concerning the mid-metabolism of tryptophan. Ch'en Shan-ming (1,2) has proved that the liver section of the rat can convert tryptophan to pyridine-carbonic acid and nicotinic acid. The Vitamin B<sub>6</sub> deficient liver section produced more pyridin-dicarboxic acid but a less amount of nicotinic acid. The pyridine-dicarboxic acid production may decrease to a normal level in the rat's liver after adding phospho-pyridine aldehyde. All these have explained that the pyridine dicarboxic acid is not the mid-metabolic product during the process of conversion from amino acid to tobacco acid. (3)

During the catabolism of tryptophan, anthranilic acid and 3-hydroxyanthranilic acid are produced. As to how the anthranilic acid converts to 3-hydroxyanthranilic acid, Fang Yu-chung and Wang Ying-lai (4) have done a series of re-

search. They extracted an enzyme which promotes hydroxylation in the cat liver's mitochondria, and they have also studied its properties. There is almost no 3-hydroxyanthranilic acid in the normal liver of the rat, but it can be very easily discovered in the Vitamin B<sub>6</sub> deficient livers of rats. The hydroxylation energy of mitochondria of riboflavin deficient rat liver is lower than the mitochondria of the normal rat liver; this explained that the hydroxylase and riboflavin have a very close relationship.

Under the condition of Vitamin B<sub>6</sub> deficiency, the conversion ability of tryptophan to anthranilic acid will not decrease (5) but the adaptation ability of oxidase enzyme will be affected. It is generally agreed that when tryptophan becomes pyridine-dicarboxic acid or nicotinic acid, this enzyme is the first step because this is an adaptable enzyme. It may be possible due to the change of the free amino acid composition in the Vitamin B<sub>6</sub> deficient liver that affects the liver protein composition when these kind amino acids are used. (6) The peroxidase action of tryptophan is inhibited by benzyl-alanine, tyrosine, cystine and cysteine. (7)

## 2. Protein Metabolism

The influence of protein deficient diet and rich protein diet on enzyme's activity in the rat's liver was studied systematically by Ch'en Shan-ming and others. (8,9) Their conclusion was that during the protein deficiency period, the activity decreasing rate of L-Leucine transaminase, glutamic acid dehydrogenase, glutamic acid and asparagine transaminase is the same as that of liver protein. After the rat is put back to protein rich diet, the enzymes' activity will increase, while the liver protein increases. The decreasing rate of enzyme activity of Glutamic acid and Alanine transaminase, Xanthine oxidase and urease were faster than the decreasing rate of liver protein, but the increasing rate becomes slower than that of liver protein after the rat is put back to protein rich diet. The changes of enzyme in tissue protein is rather great. If 4 percent of methyl sulfonamide acid is added to the diet, the rat's appetite will be increased, its body weight will decrease, the enzymes activity will increase quickly but the leucine enzyme and tissue protease will have only little change. The same molecular weight cystine, bile salt or lysine could produce similar effect as the methyl sulfonamide acid did. (10) This explained that the effect of

methyl sulphonamide acid was caused by its whole molecular weight.

Tung Ch'ang-yu has observed that the enzyme activity of cystein desulphurase in the regenerated liver is higher than in the normal liver in white rat experiments. If one group of rats is fed with 5 percent casein diet (low protein diet) and another group with 18 percent gelatin alba diet, the enzy-activity of both groups will decrease. This explained the quality and quantity of dietary protein contents could influence desulphurase in the liver.

In the study of urea secretion in rats, Ch'en Shan-ming has discovered that the amount of urea secretion in Vitamin B<sub>6</sub> deficient rat is the same as in the normal rat. Even after intaking a large amount of protein or amino acid, there is still no obvious change. This shows that in Vitamin B<sub>6</sub> deficient animals, the transaminase is lower than normal, but it is enough to meet the emergency demand of the body.

Wang Ch'eng-fa and Ch'en Ch'un-ming (11) have observed that the appetite and body weight will decrease in riboflavin deficient adult rats, and if there is a lack of protein in the diet they will decrease more. If the rats are given again a diet containing riboflavin, the protein regeneration rate is rather slow. This explains that riboflavin is very important to protein synthesis in animal body. Wang Huai-chou (12) and others have also detected poor appetite and loss of weight in young rats.

Fan Wen-hsun (13) and others have studied the influence of starvation and regular feeding to the nitrogen balance of rats. They concluded that the body weight and organ weight will not be obviously changed by protein contents in recovered diet, but nitrogen balance and liver nitrogen are related to dietary protein contents. The protein contents in the feed before starvation has no remarkable influence to liver nitrogen after protein diet is restored.

In the area of comparative physiology, Ch'en Hui-li and Lie Liang have observed transaminase action in the liver and brain tissue of five kinds of vertebrates (white rat, pigeon, turtle, toad, and the bream fish). They discovered that among all the amino acid used, the transaminase action of asparagine and alanine is the highest. There are big differences in the arginine and ornithine

transaminase action in the liver of these five vertebrates. They also proved that the group amino acid has a different transaminase action in the liver of white rat and toad.

### 3. Vitamin Metabolism

Very little study has been done in the area of vitamin metabolism. The main research work done so far concerned the physiological synthesis of Vitamin C. As for the effect of vitamin to amino acid and protein metabolism, it has already been introduced previously.

The experiments on the physiological synthesis of Vitamin C done by Chang Yu-tuan and Tung lin, (14) explained that only in the mitochondria of rat's liver there is a clear oxidase activity of the L-glucuronic acid- $\gamma$ -lactone or L-galacturonic acid- $\gamma$ -lactone. They also did some primary work on the isolation and purification of this enzyme agent. Under the condition of riboflavin deficiency, the oxidation of L-glucuronic acid- $\gamma$ -lactone in the liver will decrease to one third 3 weeks later. During this time, the L-antiscorbutic acid of various tissue and the secretion in the urine is the same as other animals. This explains that riboflavin and the oxidation of L-glucuronic acid- $\gamma$ -lactone are very closely related. (15)

### 4. Hormone Metabolism

During the study of tissue therapy conducted by Sun Chia-shou and others, (16) they discovered that rat tissue extract solution can decrease the antiscorbutic acid action of the adrenal cortex. The time of decrease action is slightly later than the AOTH action; this action will not happen in the rat whose pituitary has already been taken out. This tissue extract solution could increase blood pressure and urinary AOTH contents, and some drugs can prevent the action of this tissue solution. According above these facts, the researchers consider that there are some thing in the tissue extract solution which could stimulate the pituitary gland to release AOTH, and this thing could possibly be a low molecular compound. (17)

By injecting AOTH into anti-scorbutic acid deficient guinea pig, an obvious increase of fat in the liver will be found. The animal's liver glycogen content was increased in the deficiency group, but there was no direct influence to the liver glycogen synthesis after injecting AOTH. (18, 19)

In experiments about the effect of Chinese drug to the adrenalectomized glycogen contents in rat liver, they discovered that "ho-shou-wu" and "yu-chu" have the similar action as the adrenocorticotrophic hormone, and also proved by paper chromatography that "ho-shou-wu" extract solution may contain a similar substance as adrenocorticotrophic hormone.

Besides, Ting T'ing and others have studied paper chromatography method to determine the free cortisone and 17-hydroxyl adrenosterone in urine. Chang Kuo-lin<sup>(20)</sup> has analysed the amount of 17-ketone cholesterol and 17-ketone cortisol cholesterol in the urine of various male and female age groups in our country.

### 5. Brain Metabolism

The purpose of biochemical research about brain function is to expose the function of nervous system in relation to its chemical composition and metabolism. Since the liberation, the biochemical research workers in our country have started scientific research on brain function. Undoubtedly, this is a good beginning, and they have obtained certain achievements.

As to the function of brain in connection with the change of chemical composition and enzymes activity under different condition, the Biochemical Research Institute of the Academia Sinica has used a mixed solution of solid  $\text{CO}_2$  and allyl ketone for rat fixation. They improved Conway's micro-diffusion instrument for nitrogen determination so that it could be used to measure 0.05-0.50 mgm of ammonia. They have determined ammonia amount in different areas of the brain of rat. The average amount of ammonia in the area which controls hearing, vision and body is 0.36, 0.36 and 0.37 mgm percent respectively, but the average amount of ammonia in mid-brain, cerebellar, pons...and other areas is up to 0.50, 0.46, 0.39, 0.40, 0.44 mgm percent respectively.

They also found out that the changes of ammonia in rat brain is very closely related to the brain's function condition. They also discovered that when there is a lack of glucose in the brain section, most of the glutamic acid is converted to asparagine, which may be changed to glutamic acid again by glucose. It was also discovered that the preservation of glutamic acid, asparagine and  $\gamma$ -threonine needs the presence of glucose and oxygen. The result of the study shows that this metabolic change may be closely related to the

metabolism of acetyl activity. (22, 23) The study of protein in cow's brain by using the methods of paper electrophoresis and dissociation staining proved that the nucleoprotein or lipo-nucleoprotein extracted from the brain are not the true compound of nucleic acid and protein as reported in foreign literature. (24, 25)

The biochemical teaching and research group of the biology department of the Peking University has collected some material on the determination of enzyme activity in brain. The brain transaminase activity will decrease  $1/3$  when the mice is induced to sleep by barbital sodium. (26) After intoxication of the mice by carbon monoxide the peroxylase activity decreases in the brain, and the peroxylase activity in the liver also decreases. (27) The amino acid kinase activity in pigeon's brain is higher than the kinase activity in its heart, but lower than the kinase activity in its liver, kidney, and intestinal mucous membrane. (28)

Brain enzyme activity changes during the stage of individual development and systemic development. The activity of various enzyme in animal brain tissue only shows in certain stages during individual development. The brain transaminase activity in the chicken embryo begins to increase quickly on 16 days after the chicks are hatched from eggs. (29) Only a little activity of acetyl-cholinesterase in the brain of mice could be detected on the 11th day of the mice embryo, and it gradually increases on the 13th day, and becomes stable from then on. In the new-born rats it increases daily after birth until the 25th day. (30) The brain acetyl cholinesterase activity could also be observed during the systemic development stage of vertebrates. For instance, the enzyme activity in fish is the lowest; it is very low in frog and toad, but it is high in birds and mice or rabbit; it is the same in reptiles (turtle and snakes) as in fish. It is very possible that the acetyl cholinesterase activity of the warm blood vertebrates is very much influenced by the various functions of the brain organ. In systemic development, the conditions are different between 6-phospho-glucose dehydrogenase and brain acetyl cholinesterase; the former is rather primitive and the latter could only be found after the systemic development stage. The activity of 6-phospho-glucose dehydrogenase is very high in frog and toad, but very low in chicken and pigeons. In dog's brain, the 6-phospho-glucose dehydrogenase activity is different according various conditions; this explains that in the



cerebellar and pons (the early developed portions), there is a higher activity of 6-phospho-glucose dehydrogenase, but in the late developed portions such as the cortex there is a lower activity of this enzyme. The development of various portions of the brain is related to the functions of this enzyme. Although materials concerning brain enzyme activity during the stages of individual and systemic development are still lacking, the theoretical significance of the experiments thus far is clearly shown.

### 6. Pathologic Metabolism

In regard to pathologic metabolism, it has already been reported in detail in the section of clinical biochemistry. Here we only introduce some of the study on tumor.

Yuan Ch'ang-kue<sup>(24)</sup> has studied the acid and alkaline phosphatase in chickens inflicted by tumor by using the tissue chemistry and biochemistry methods. He discovered that the alkaline phosphatase activity of GRCX/15 sarcoma in the tumor is higher than that in other organs except the (liver and kidney) of the host. The acid phosphatase activity in Rous sarcoma is lower than that in other organs. The malignant change of the tumor has no clear relation with these two enzymes.

The biochemistry department of the Chinese Medical Science Institute has determined the activities of certain enzymes in the normal tissue and several transplanted tumor tissues in young rats by using the micro- and ultra-micro enzyme activity determination methods. These enzymes include arginine enzymes, tissue protease, glutaminase, glutamic acid acetyl transaminase, triphosphatase, acid phosphatase, alkaline phosphatase, nucleio-phosphatase, nucleio-nuclease and deoxyo-nucleio-nuclease. The results showed that the enzyme activity patterns in normal lymphatic tissues such as spleen, lymph gland and thymus are similar but different from the activity pattern in the liver. The basic enzyme activity pattern in the tumor tissues are the same. This is especially obvious in the ascitic type lymphocytic leukemia and lympho-sarcoma. Generally, enzyme activity in solid type tumor tissue is higher than in the same tumor tissue which produces ascitic tumor cell.

They discovered that the patterns of free amino acid distribution in the white mice's lymphatic tissue such as

the thymus, lymph gland and spleen are similar, but different from those in the liver, lung and heart, the patterns of distribution in the latter three being different from one another. The free alanine in productive lympho-sarcoma is higher than in normal lymphatic tissue, but there is a decrease of asparagine. The alanine will increase in the transplanted lympho-sarcoma, but the glutamic acid will decrease. The free alanine is higher in tumor lymphatic tissue than in normal lymphatic tissue, especially in the liver.

During the study of enzyme activity change in the tumor developing stage, they discovered that the glutaminase activity is higher in the tissue of productive lymphosarcoma, transplantative lymphosarcoma and Ehrlich ascitis carcinoma than in normal lymphatic tissue. The activity increases as the tumor grows.

The Biochemical Research Institute of the Academia Sinica has conducted studies of early diagnosis of cancer. They have determined the amount of 17-ketone cholesterol secretion in cancer patients and liver disease patients. They found out that the amount of urinary 17-ketone cholesterol is less in hepatoma, liver cirrhosis, and hepatitis patients than in the normal individual. They made further studies of the method to distinguish liver cancer from other liver diseases. Besides, they also did some study on the relationship between serum tryptophan and other acids, but this relationship cannot be used for the diagnosis of tumor.

They have analysed the serum of patients by certain analytic patterns, and discovered that there is a definite difference between the serum pattern of hepatoma patients and that of cirrhosis patients. This discovery has a considerable value to the diagnosis of these two disease. Since this pattern is not a unique pattern of hepatoma, this method only has a reference value to clinical diagnosis and cannot be used for early diagnosis of hepatoma.

For the last ten years, the research workers in the field of biochemistry in our country have done many studies on metabolism, and undoubtedly they have gained great achievements in their study. But owing to the fast development of biochemistry recently, there are still some blanks in this field, such as the important problem of nucleic acid metabolism, the effect of radiation on metabolism.

etc. Even though we have an excellent beginning in hormone metabolism and brain biochemistry studies, we should still go forward in the field of science, and learn the advanced experiences of the Soviet Union and other countries.

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## RESEARCH ON PROTEIN

Following is a translation of an article by Liang Chih-ch'uang entitled "Tan-pai-chih ti yen-chiu kung-tso (English version above), in Ch'ing-kuo Chien-kuo Shih-chou-nien I-hsueh K'c-hsueh Ch'ang-chin Lun-wen-chi (Collected Essays on the Achievements of Medical Science in Celebration of the Tenth Founding Anniversary of the Nation), Vol 1, compiled by the Ministry of Public Health, Peiping, 1959, pp 135-138.

Before the liberation, little work had been done in the field of protein research in our country. Even if there was any such work, it was completely dissociated from the demand and the need of the masses. Since the liberation, however, much progress has been made in biochemistry research under the encouragement and support of the party. Among other things, work on protein research has been more emphasized and its achievement more impressive.

First of all, more work has been done in the studies of plasma protein. Such studies are closely related to the practical need. Furthermore, studies on tissue protein are also systematic and conclusive. Research effort in these two phases is indeed worth mentioning.

In the area of plasma protein research, some units have obtained good results through such kinds of studies as the preservation of non-specific plasma and liquid plasma; and the production of dehydrated plasma.<sup>(1)</sup> The researchers have confirmed that the non-specific plasma made by heating the pig plasma is non-toxic to most animals, and therefore it is used clinically for the prevention of hemorrhagic shock. However, the antigenicity is not completely destroyed. This kind of work is at present being continued by the Blood Transfusion Research Department of the Chinese Medical Science Institute.

Lin Kuo-kao, Wu Wei and other coworkers used three different methods in their study of the change in antigenicity. These methods were: alkali alteration, glucose heating,

urea and thio-urea alteration. The result indicated that antigenicity could be comparatively reduced by applying the alkali method, but there was some toxicity. On the basis of the alkali method, they<sup>(2)</sup> further studied five different physical-chemical methods in an effort to reduce the toxicity. Among them, the more fruitful one was the method of alkali alteration followed by formaldehyde treatment at high temperature in short duration, again followed by low temperature in long duration and finally by glucose heating treatment. This product could improve the condition of mice which had become toxic. It was also proved to be good in the experiment of sensitization of the mice.

The Wu-han Biological Product Research Institute has also greatly improved the method of producing non-specific plasma. Their products have been used for clinical purpose. But short-comings still exist. The work of obtaining a single pure plasma protein in the study of correlation between alteration and antigenicity is now being conducted at research laboratories in the Chinese Medical Science Institute.

Wang Lin-fang and Liang Chih-sh'uan used various amount of radon radiation to treat bovine serum albumin solution in order to observe the effect of  $\gamma$  rays on the chemical and biological specificity of protein. The optical activity of natural serum albumin changed little in different PH. After radiation, optical activity markedly increased in slightly alkaline or acidic condition. Viscosity after radiation, however, changed little. Using its reaction with S<sub>2</sub>S cysteine as a standard, the energy of reaction showed an increase after radiation. When the radiation dose surpassed 5,000 mch, molecules split and molecular weight was reduced to one half of the original. When protein underwent changes, its biological specificity also changed markedly. Using precipitation titration as a technique, the protein antigenicity was observed to have decreased after radiation. The above-mentioned physical chemical nature was closely related to the total dose of radiation. But they had little correlation with concentration.

As to the better application of human plasma, the Experimental Medical Research Department of the Chinese Medical Science Institute employed the method of low temperature ethyl-alcohol fractionation on human plasma. They also conducted the study of using zinc ion in human and bovine plasma protein separation.

Wu Wei<sup>(3)</sup> and his coworkers conducted the research of oxy-polymer gelatin as a substitute for plasma. They made the oxy-polymer gelatin solution according to the method advanced by Pauling and Campbell. The average molecular weight approximated 20,000. Owing to the low molecular weight, its duration in blood vessel was comparatively short. After one hour's intake of this material, what was left represented one third of the total intake amount. This product should not be considered as a satisfactory material for plasma expander. However, it had certain definite clinical use for the prevention and cure of hemorrhagic shock. In this direction, another study was conducted by Fan Chi-shou<sup>(4)</sup> and his coworkers who had made a certain fiber albumin sponge and fiber albumin membrane from pig plasma and had studied their biological and chemical nature as well as their antigenicity.

Parenteral nutritional solution made by protein hydrolysed product is being studied. This is a mixed solution containing many kinds of amino acids. This solution, which may be injected through the veins, is proved to be useful to patients before and after surgery and to those who suffer from digestive disorder. Research work in this area, started in 1954 by some units, is now being conducted by the Blood Research Department of the Chinese Medical Science Institute. The researchers have been successful in producing a synthetic compound by using casein albumin, unwashed red blood cells and soy beans as raw material. In this respect, Wang Yiu-chang<sup>(5,6)</sup> has advanced a new method of using mildew fermentation to make casein albumin and soy bean hydrolysed product. Comparing with pancreatin hydrolysis, this new method requires no antiseptic agent and less time in completing hydrolysis.

So far as the research of cancer protein is concerned, Wang Shih-chung and Keh Yun-chin have succeeded in isolating and purifying Bence Jones albumin from the urine of multiple myeloma patients. On the basis of this protein, they conducted studies on immunology and amino acid composition. They have discovered that this protein does not cause rabbit to produce precipitable antibodies. In terms of amino acid composition, this protein differs from those found in other parts of the human body.

Research laboratories of the Chinese Medical Science Institute have conducted studies on five different methods of antigen purification in an effort to deal with Ehrlich ascitis carcinoma. Some results obtained so far indicate



that antigenicity exists in globulin part. On the other hand, the antibody characteristics are found in the area of  $\gamma_2$  globulin. Animal experiments have given ample evidence that using the soluble portion of the Ehrlich ascitis carcinoma in physiological saline as antigen to immunize mice, can suppress the growth of a particular type of tumor cells. If the mouse is passively immunized by giving it antiserum, its life time is on average prolonged twofolds. In treatment of the localized skin cancer, application of anti-serum injection within six days after the cancer cells are attacked will have some good effect.

Ever since 1953 workers in our medical field have developed a high interest in studying Pavlov's theory. Workers in the biology and biochemistry field are also inspired to pay more attention to the biochemical nature of the nervous system. In the aspect of protein studies, Chao Tien-chin<sup>(7)</sup> and associates have conducted studies in the isolation of nucleic protein and lipid-nucleic protein in the brain and their structural combination. As a result, they find that all these proteins have been formed during the course of isolation. They are not the true combination product of nucleic acid and protein. Li Tsai-p'ing<sup>(8)</sup> and his coworkers, on the other hand, have succeeded in isolating pure albumin from the cow's static nervous system and further proved that it could possibly be the same material as serum protein.

In immune chemistry, there were also some achievements, though less conclusive. Tao Yi-hsun<sup>(9)</sup> and his associates studied electrophoretic analysis of sera from cow, chicken, goose and sheep. They found that all these sera exhibit fifteen boundary belts.

T'ao Yi-hsun<sup>(10)</sup> and his coworkers also studied the biochemical aspect as well as the immunological aspect of the soluble antigen of the female and male *Schistosoma Japonica* and its eggs. They found that in the antigen solutions there were many proteins and the antigen solution exhibited different antigenic property. This finding clearly indicates that during diagnosis the difference in the degree of sensitization and specificity in many antigenic solutions is due to the variety of the composition of the antigenic solutions. Furthermore, research effort should be made to tackle the problem of isolation and purification of antigen solution. This work is important for diagnostic purpose.

In the study<sup>(11)</sup> of the immunological nature of the

low degree antibody, it had been proven that low-degree antibody existed in the immune serum of domestic rabbit. This type of antibody cannot unite with antigen alone and thus cause precipitation. But they can, in the presence of complete antibody, unite with antigen and cause precipitation. When it precipitates, it possesses specificity. The writer of this paper is of the opinion that the low degree antibody is a by-product in the course of the formation of the complete antibody. Hsieh Yen-po, (12) by using the low temperature alkali treatment, has produced pure antibodies from the egg white albumin precipitin. This precipitin is again induced by the egg white albumin antigen. The purity can be as high as 96 percent. The electrophoretic behaviour is the same as  $\gamma$  globulin in rabbit serum. The diffusion coefficient is somewhat larger than  $\gamma$  globulin.

In the area of tissue albumin, research work has been more systematic and conclusive. Tsao Tien-chin (13,14,15) and his associates made crystallized myoglobulin from various muscles. They determined its physical-chemical properties, its structure, molecular weight and molecular symmetry, electrophoretic velocity and stability. They further studied the amino acid composition of several muscle globulin and the manner of linkage of their amino end as well as C(16,17) end. They also altered the groups on the side chain and observed the change in activity, solubility and stability. (18) As a result, they found the asymmetrical nature of these myoglobulin. They unite in neutral solution and dissociate in salt solution. Muscle globulins coming from different sources have different molecular weight. They are all stable, and able to stand high temperature for a short duration. In chemical composition they have more polar groups than other proteins. They all lack proline. Possibly, they have no side group on the N end. On the C end, the group is  $\text{O}_6\text{H}_4\text{COOH}$ .

Replacing its amidine group or the guanide group will greatly influence their binding ability. Acylation does not influence very much on their binding ability.

They have proposed a method on the preparation of nucleic-myoglobulin. They found that the nucleic acid portion was pantosan nucleic acid. From electrophoretic findings, (19) they could show that the bond between nucleic acid and protein is rather loose. (20,21,22,23)

They have also isolated a new protein from muscle and purified it. This protein has a precipitation coefficient

of 6.3. Molecular weight approximates 140,000.

Besides, they have studied the structures<sup>(24,25)</sup> of the motor fiber albumin and isolated a crystalline product from alcohol treated rabbit meat powder. This product is the combination of a calcium and magnesium salt of phosphoric acid on the one hand, and protein on the other. In addition, they have also studied the amino acid composition.<sup>(26)</sup>

In connection with the method of the determination of protein, the following work has been done: fractionation electrophoresis and paper electrophoresis have been widely used by protein biochemists and clinical biochemists for the determination of purity, for the synthesis of pure protein and for the study of serum protein under a pathogenic disorder.<sup>(27-31)</sup> Amino acid determination by paper chromatography<sup>(32-34)</sup> and by microorganism assay<sup>(35-37)</sup> have gained much improvement. Beside, there are improvement on Hepp's osmometer. Studies have been done in determining the diffusion coefficient by using Antweiler electrophoresis device.

The above brief introduction has made clear to us that our work in the field of protein research is still quite insufficient. There are many blanks. From the medical point of view, future research emphasis should be directed toward the following areas: protein linkage, its energy correlation, radiation effect on protein, study on protein under pathogenic disorder, theoretical relationship between connective tissue protein, lipid-protein and nucleo-protein.

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## STUDIES ON ENZYMES

Following is a translation of an article entitled "Mei ti yen-chiu" (English version above), by Li Shih-o, in Ch'ing-chu Chien-kuo Shih-chou-nien I-hsueh K'o-hsueh Ch'ang-chiu lun-wen-chi (Collected Essays on the Achievements of Medical Science in Celebration of the Tenth Founding Anniversary of the Nation), Vol. 1, compiled by the Ministry of Public Health, Peiping, 1959, pp 138-142.

Enzyme is an active protein in the living body. It has the effect of stimulating all chemical reactions in the body such as oxidation, reduction, hydrolysis, phosphorylation .. etc. It has a very close relationship with metabolism. Since the liberation, we have gained considerable achievements in the area of enzyme study, and studies on the isolation and purification of enzyme and its properties and mechanism. This has built an excellent foundation for future researches.

### I. Basic Theoretical Studies of Enzyme

The metabolism of enzyme system is very complicated. For instance, the oxidation of 1 gram-molecule glucose into  $\text{CO}_2$  and  $\text{H}_2\text{O}$  must through the reaction of more than 20 different kinds of enzymes, all of which have individual characteristics. In the study of enzyme action during metabolism, one must learn the mechanism of all the enzymes. Therefore, the basic theoretical studies are very important.

1. Enzyme systems related to the oxidation of organisms. This is a very complicated and also very important enzyme system. Owing to the efforts of our biochemical workers, we already have an excellent beginning in this field. In the area of enzyme systems related to cytochrom, Chou Ch'en-lu and Wu Chin-yung<sup>(1-3)</sup> have proposed a method of using the two enzyme systems to fighting for one common factor. By this method, the relationship between these complicated enzyme systems may be found out. By this method, they have also found out many enzyme systems related to cytochrom in several animal tissues, including the coenzyme-1 oxidase system, succinic acid oxidase system,

choline oxidase system and  $\alpha$ -glycerophosphate oxidase system; all these enzyme systems contain a common "intermediate factor." The choline oxidase and succinic acid oxidase systems both contain cytochrome b, but the reduced coenzyme I and  $\alpha$ -glycerophosphate oxidase systems have no relation with cytochrome b. They also proved that the "hydrolytic coenzyme I cytochrome C reduction enzyme" does not truly exist in the cardia muscle as the literature said.<sup>(4)</sup>

Wang Ying-lai and others<sup>(5)</sup> have proved that the bile acid salt's inhibitory action to succinic acid dehydrogenase and cytochrome oxidase system is much lower than its inhibitory action succinic acid oxidase. They suspected this bile acid salt action seems to be between cytochrome B and C. Chou Oh'an-lu and Li Wen-chieh<sup>(6-9)</sup> have proposed a new method for preparing animal cardia muscle and yeast cytochrome C, and also did some observation on the analytic method on the N-end amine acid of cytochrome C and the pure yeast cytochrome C.

As for succinic acid dehydrogenase, Wang Ching-yin, Chou Oh'an-lu and Wang Ying-lai<sup>(10,11)</sup> have conducted further studies in this field. They have isolated a purer enzyme agent from animal cardia muscle, and its activity is twice as high as the enzyme agent reported about the same time in foreign literature. The isolated enzyme contains riboflavin and iron.

Wu Ching-yun and Chou Oh'an-lu<sup>(12)</sup> have done some comparative study on the ability of various xanthine dehydrogenase by using different hydrogen receptors in the nitrogen metabolism of the animal's liver. The results shows that only xanthine dehydrogenase of the animal possesses the ability of using coenzyme I as a hydrogen receptor. Besides, Chou Oh'an-lu<sup>(13)</sup> has discovered an enzyme from the cow's liver which has a stimulating action somewhere between coenzyme I and coenzyme II as it transfers hydrogen. It acts like an alkaline pyridine nucleic acid transaminase, and is proved to be a new enzyme.

A further step is to study the mechanism of the complicated enzyme systems, a very important research. In this area, they have studied the  $K_i$ 's combination constant for two enzyme acting at the same time,<sup>(14)</sup> the mechanism of xanthine dehydrogenase,<sup>(15)</sup> and the combined mechanism of glutamic acid dehydrogenase and coenzyme I and II.<sup>(16)</sup>

Besides, Chou Ch'en-lu and others (17,18) have conducted some studies about poly-phenol oxidase, discovered some organic solutions which could increase the activity of oxidase, and also did some observation on the coenzyme factor of this enzyme.

2. Enzyme systems related to phosphorous metabolism: In the area of phosphatase, Shen Shao-wen and others (19-23) have studied the effects of isolation, Ph, temperature, enzyme concentration, inhibitory agents of the yeast alkaline phosphatase upon the activity of enzyme. The inhibitory action of enzyme by sulfamide is found reversible, but not competitive. They considered that the action of sulfamide is due to its pyridine. They also conducted a similar study on the green lentil sprout phosphatase, and discovered, that some of the inorganic ions such as Zn, Cu, F have very strong inhibitory action on enzyme activity. Besides, Peng Chis-lu and Wang Ying-lai (24) have studied the isolation and purification of the acidase of soybean and its property. They also did some observation of its mechanism.

In the area of phosphoaminase, Li Shih-s and Chang Chih-ping (25,26) used a series of synthetic acetyl-phosphoamine group compounds as a substrate. They studied the property and distribution of this enzyme in vivo, and discovered that its highest activity is in the spleen of the rat tissue.

In the study of the characteristics of phosphoprotein phosphatase, they explained that this enzyme does not seem to be a specific enzyme. The action of phosphoric acid from phosphoprotein may be due to the sum of the chain activity during the hydrolysis of various phosphoprotein such as O-P, N-P, and di-phosphotidate chains. (27)

3. Enzyme systems related to the nucleic acid metabolism: Sun Ch'ih and Wang T'e-pao (28) have found that in the spleen of rats, mice and chicken, there is a high activity of ribodesecose-phosphate aldehydease. After isolating this enzyme from the rat's spleen, they proved that its action is reversible. Wang T'e pao (29) has discovered a new nucleoside hydrolase. This enzyme has an effect on pyridine nucleoside, but has no effect on purine nucleoside, or pyridine deoxidize nucleoside. This explained that there has no nucleoside-glucose nucleoside changing to glucose in the same enzyme solution. The enzyme activity of dissociate non-xanthine acid may be found in animal's liver, kidney tissue, and the highest activity was found in the



rat's liver. They also did some studies on the properties, isolation and purification of this enzyme.(30) The nucleoglucose-nucleodase of the pig's pancreas and cow's pancreas have no effects on non-xanthine acid.(31)

4. Other enzyme systems: While the study of tryptophan metabolism was going on, Fang Yu-ch'ung and Wang Ying-lai(32) conducted at the same time studies on the isolation, purification and the properties of anthranilic acid hydroxylase. That cyanide can increase the hydroxylation action may be due to the reason that it inhibits the oxidation and reduction of coenzyme I. They have proposed a chromatography for determining 3-hydroxyl anthranilic acid and anthranilic acid.

Ku T'ien-chic and others(33) have prepared a specific glutamic acid de-carboxylase from the E. Coli to determine the activity of L-glutamic acid and serum transaminase. The results of the experiments Chu Wei-tung and others have proved that the serum transaminase activity may be used as an index in the acute antimony intoxication, but not in chronic intoxication; the transaminase in vivo may gradually produce adaptability to antimony.

Ch'en Chun-piao and Shen Shan-ch'ung(33) have isolated and purified monnitol-isomerase from the single bacillus aureus of the string bean. They proved that its reaction is reversible and the products is fruit sugar, which has an inhibitory effect on the enzyme activity. This explains that this enzyme action needs HS.

Shen K'ung-mou and Shen Yun-kang(34) in the study of phosphorylase, have proved that the inhibitory action of amylase on phosphorylase activity was due to the result of the dissociation of amylase by phosphorylase.

Besides, they also studied about the effect of pancreatin,(35) and the effect of red blood cell to the reduction action of normal iron hemoglobin.(36)

## II. The Properties and Distribution of Enzymes In Vivo Under Different Physiological Conditions

On the study of the physiological effects of enzymes, we must study the properties and distribution of enzymes under normal and various physiological conditions and also their activity changes during the individual and systemic development stages of the vivo. The following is a des-

scription of our work concerning this subject:

1. In the area of individual and systemic development: Li Shih-o and Ho Yen-sheng<sup>(37)</sup> have observed the phosphoprotein phosphatase changes during the chicken embryo development stage. They concluded that the highest enzyme activity in the yolk sac occurs on the 7th day; in the chicken embryo, on the 4th day. As for the various organs of chicken, the highest activity occurs in the brain. This enzyme activity may be connected with the utilization of phosphoprotein of the egg. Chu Lo-ch'ang<sup>(38)</sup> has studied the central nerve system transaminase activity changes in the chicken embryo development stage, and also determined this enzyme's activity in the central nerve system. Wu Kuo-li<sup>(39)</sup> has studied the cholinesterase activity changes in the brain of the mice's individual development. Besides, the Biological Department of the Peking University has conducted a series of enzyme activity determinations in the various portions of the brain, and also observed their activity changes in the individual and systemic development period.

By using the tissue chemistry and biochemistry methods, Wang Kun-jen and others<sup>(40)</sup> have studied the appearance of peptic enzymes in several peptic glands during the animal developmental period. Their result concluded that the pancreatic amylase is the same from the time of birth till maturity, the others such as ptyalin, pancreatin and pepsin reach their maturity level 2-4 weeks after birth. Several peptic tissues have shown a very strong activity of the adenosine triphosphase.

2. Enzyme distribution in vivo under normal and various physiological conditions: Lu Yen-lin<sup>(41)</sup> used the method of tissue chemistry to observe the embryo of mice. On the 10th day, when the embryo cell growth is very active, the alkaline phosphatase activity is very high. As in the various organs of the grownup mice, the highest activity is in the small intestine epithelium and in the nephrons of the kidney. But in the adrenal cortex, enzyme activity only shows in the male mice. Lu Yen-lin and Yao Ming-hsin<sup>(42,43,44)</sup> have observed that long-term injection of female hormone or transplacation of the ovarian cyst may cause this enzyme disappear from the adult male mice' adrenal cortex.

Ku kuo-yen and Yao Tseng-hsu<sup>(45)</sup> have used the combined methods of tissue chemistry and biochemistry for the

study of phosphatase activity and distribution changes during the stages of growth and degeneration of rat's mammary gland. According to the conditions of change, they pointed out that the acid and alkaline phosphatase have a close relation with the mammary glands growth, energy dissociation, production of milk and the dissociation of degenerated tissues.

Yao Hsin and Cheng Chu-yin(46) have observed that during the fly's abnormal generation stage, the acid and alkaline phosphatase activities have changed markedly. These changes were closely related to tissue dissociation, tissue development and organ degeneration. The properties of these two enzyme are the same as in other animal tissues.

The above studies have supported the theory which considered that the phosphatase is related to secretion, absorption and cell dissociations.

Besides, Li Chin-hua and Chu Sheng-kuang(47) have studied the distribution of amino acid enzymes in the various tissues of pigeons, and found that liver was most active. They also discovered that the enzyme activity in the rat's liver was not influenced by protein contents in the diet.(48)

Owing to the fact that enzyme is closely related to metabolism, studies of enzymes under pathological conditions may be of reference value to the study of the mechanism of disease. More details in this respect appear in the special report on metabolism.

### III. Clinical Use of Enzymes

The determination of urinary and blood enzyme activity changes in patients may help clinical diagnosis. This has already been discussed in the section on clinical biochemistry. A great deal of study has already been made about amylase(49,50) glutamic-oxalo-acetic acid transaminase(51) and cholinesterase.(52) Other studies were concerning the determination of the activity of peroxidase,(53) amylase,(54) alkaline phosphatase,(55,56) and glutamic-oxalo-acetic acid transaminase.(57)

The above shows that the biochemical researchers in our country have done a considerable amount of work in the field of enzymes, and gained certain achievements. Although the purpose and direction of these studies were not

well defined, they have definitely built a good foundation both from the technical and theoretical point of view. From now on, we have to follow up the development of biochemistry science, and must study more about the structure, and mechanism of enzymes. Furthermore, we have to do more studies on enzymes in relation to the nerve system, nutrition and hormones. Through this study, we will find out how to control enzyme growth and enzyme activity changes under pathological conditions. This will not only help us understand normal physiology, but also throw light on the prevention and treatment of diseases.

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## ACHIEVEMENTS IN THE STUDY OF HORMONES

Following is a translation of an article entitled "Chi-shu yen-chiu ti cheng-chiu" (English version above) by Ting Yen-chieh, in Ch'ing-chu Chien-kuo Shih-chou-nien I-hsueh K'o-hsueh Ch'eng-chiu Lun-wen-chi (Collected Essays on the Achievements of Medical Science in Celebration of the Tenth Founding Anniversary of the Nation), Vol 1, Compiled by the Ministry of Public Health, Peiping, 1959, pp 142-144.]

In the past, the emphasis of the study of hormones was on their physiological actions. Recently, we have employed biochemical methods in the study of hormonal endocrine mechanism, and some of the results have been used clinically.

In the biochemistry of hormones, the most important problems concern hormonal effect mechanisms (especially its relationship with the enzyme) and the structures of hormone itself, its energy, biological composition, and metabolic changes etc. These are the topics at which our efforts will be directed in the future.

Following is an introduction of our achievements in hormone studies.

### I. Pituitary Hormones

#### 1. Adrenocorticotrophic hormone (ACTH):

(1) Hormones' influences to metabolism: Ch'en Shuang-ch'i (1,2) and others have reported that after injecting ACTH to a group of normal guinea pigs and comparing them with the control group, they found a slight increase of blood sugar and liver water, but no change in liver glycogen, fat and nitrogen contents. After injecting ACTH to the Vitamin C deficient animal, they found a decrease of blood sugar and liver water contents, no change in liver glycogen, a decrease of liver nitrogen content and a marked decrease of liver fat contents. In these two groups of animals, there is no direct influence to the liver glycogen composition after the injection of ACTH; the liver glycogen



will increase after intake of a large amount of glucose, but the increased amount in the Vitamin C deficient group is less than in the normal animals.

Besides, there were many reports concerning the clinical application of ACTH.

(2) Pituitary stimulating factors: The biochemical research and teaching group of the Medical School of Wu-Han University(3,4) discovered a substance which stimulates the pituitary gland secretes ACTH, and this substance was found in many tissue extract solutions. The researchers named it pituitary stimulating factor. After injecting the solution which contains this substance, the blood ACTH level in the animal will increase first. Later the adrenal antiscorbutic acid will decrease (this is an index to the activity of adrenal cortex secretion). In guinea pig experiments, it causes a decrease of cholesterol in the adrenal gland, and an increase of the 17-Ketone cholesteroid in the urine. There is no effects on the hypophysectomized animal because the drugs could cut off the conducting path from hypothalamus to pituitary gland.

The chemical structure of this substance is still not clear; we only know that it is easily dissolvable, dissociable, and heat stable (130-140°C). After oxidation, it may be absorbed by chicken protein which may also be washed off again.

2. The action of other pituitary anterior hormones: Shen Chi-Ch'un(5) has studied the influence of cow's anterior pituitary tissue extract on the pancreatic Zn contents in white rats. The results showed that the pancreatic Zn content really increases after the animal received the injection, and also that the pancreatic island of Langerhans cell was enlarged and its granules were increased (this means the function is very active). Mr. Shen considered that this may be due to the action of the additional pancreatic Zn content which may prevent the release of insulin.

3. Bioassay of the posterior pituitary hormone: Wu I-min(6) and others have reported that by using large white rats' uterus for determining the effects of posterior pituitary hormone preparations, they assured that the success ratio was higher in the preadolescence animal. This denied previous reports based on adolescent animals. They also improved the method of vagina smear from the animal. Re-

cently, the author(7) has reported that by using drug treated uterus, good results are obtained in both pre-adolescent and adolescent stages. The researchers suggested the use of this method instead of the method of vaginal smear.

## II. Adrenal Cortical Hormone

1. The chemical composition of cortin: The change from natural steroid substance to cortin stage at the 11th carbon molecule plus hydroxyl is a very difficult procedure. Hsieh Hsuen-hsien has reported that to use micro-organism *Rhizopus nigricans* may promote the oxidation of steroid to become 11- $\alpha$  hydroxyl steroid; this is a very easy method and the amount of its products is also high (about 82 percent).

2. Cortical hormone and glycogen composition: When NaCl is given to adrenalectomized young mice, the glucose changes to liver glycogen. From the result of experiments, the cortical hormone has no direct influence on the change from glucose to glycogen. From these results the author concludes that the examination of liver glycogen storage for determining adrenal cortico-glycogenic hormone (as reported in literature) is meaningless.(8)

Wang Fu-chou(9) has reported that when 1 percent NaCl solution is given to the adrenalectomized rat, the liver glycogen storage ability is recovered, but it is still unable to restore the storage ability to normal level. The muscle glycogen storage ability will decrease. The liver glycogen storage ability will completely recover if the rat is given cortical hormone. The researchers also observed that if the adrenalectomized animal is allowed to take 1 percent NaCl solution freely, its life span will be longer than what the literature stated. The researchers found that the 1 percent NaCl solution can be used to prevent early acute death, and cortical hormone can be used to prevent late chronic death.

3. The metabolism of adrenal cortical hormone and the secretion of its products: Ting T'ing in his experiments on adrenalectomized young rats, proved that a specific nicotine may decrease the speed of the destruction of cortin, and therefore prolongs the glycogen storage time in the liver.

The determination of cortical hormone and its products from metabolism, Ting T'ing studied the paper chromatography for determining the urinary free cortin and 17-hydroxyl

corticosterone and the bio-assays of sodium accumulation steroid, Sun Chia-shou(10) reported the urinary secretion amount of methyl alcohol cortico-steroid, and suggested that the determination of this substance is an excellent method for the examination of adrenal cortical function. Chang Kuo-lin(11) reported the urinary output of 17-ketone steroid and ketogenic cortico-steroid from people of different age groups in our country, and pointed out that it is different from that of the foreigners. Wang Sung-I(12) and others reported that the urinary output of methyl alcohol cortico-steroid, 17-ketone steroid and ketogenic cortico-steroid from workers working in high-temperature shops has no or only slight difference from that of normal individuals. Besides, there are many clinical reports concerning this matter.

4. The influence of Chinese drugs: Li Li-chun(13) discovered that the "ho shou-wu" extract solution contains a similar substance as adrenal cortical steroid hormone. Sung Oh'eng-yu and others studied jen-sheng(14) and "ching-chia"(15) and their effects to adrenal cortex. In addition, they have reported using "kan-ts'ao" in the treatment of addison disease.

### III. Other Hormones

1. Adrenal medullary hormone: Sun Chung-peng and Chang Hsin-an(16) have did some preliminary studies on the dog which have gone through both adrenal-medullarectomy and hypophysectomy operations. They studied the effect of this hormone to the blood sugar, blood calcium and blood oxide in dog under anesthetic and awake conditions.

2. Female Hormone: Lu Yen-ling(17) reported that injection of Estrogen may cause the disappearance of phosphatase activity in the adrenal cortex of male mice, and also has an inhibitory action on the testis and scrotum.

3. The hormone from pancreatic  $\alpha$  island: Hsu Hung-ta(18) reported that injection of oxidized cobalt into rabbits may cause destruction of the  $\alpha$ -cell of pancreatic island, and a deficiency in the regulating ability of blood sugar. These rabbits show a marked and prolonged increase of hypoglycemia reaction after receiving insulin injection in comparison with other rabbits which did not receive any cobalt. Therefore the author supports the theory that the  $\alpha$ -cell has a certain effect on the regulating ability of

blood sugar.

4. Thyroid Hormone: Wang Sung-I(12) and others reported that the P.B.I. content in workers working in high temperature shops is lower than that in normal individuals, mostly at a minimum within the normal range. In the study of the treatment of schistosomiasis, they discovered that the thyroidectomized or those on anti-thyroid drugs will have an increased tolerance of antimony toxicity. If the patients are given thyroxin or their metabolic substance is increased, their tolerance of antimony toxicity will decrease. (19,20)

#### IV. Hormone Preparations

At present we could produce many hormones in our country. The Chemical Engineering Department of the Shanghai Pharmaceutical Laboratory alone can produce several kinds of protein and multiple peptides hormones such as insulin, adrenal corticotrophic hormone and pitocin. Some work has been done on the isolation, purification and increase of its products. Hsu Chung-chia and others succeeded in the preparation of ACTH from pig's pituitary gland. Besides, the Biochemistry Research Institute of the Academia Sinica and the biochemistry teaching and research group of the Peking University(21) have composed pitocin according to available literature. This is but a beginning of the study in multiple peptide.

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## ACHIEVEMENTS IN VITAMIN RESEARCH

Following is a translation of an article entitled "Wei-sheng-shu yen-chiu ti cheng-chiu" (English version above) by Ling Yen-chieh, in Ch'ing-chu Chien-kue Shih-chou-nien I-hsueh K'o-hsueh Ch'eng-chiu Lun-ven-chi (Collected Essays on the Achievements of Medical Science in Celebration of the Tenth Founding Anniversary of the Nation), Vol 1, compiled by the Ministry of Public Health, Peiping, 1959. pp 145-149.<sub>27</sub>

Most of the vitamin research in our country was limited in the field of nutrition, and more studies were done on antiscorbutic acid, riboflavin, carotene and nicotinic acid. As far as biochemistry is concerned, only little work has been done concerning the structure, effects, mechanism, biological composition and metabolic changes of vitamins, and most of these work were done after the liberation. Following is an introduction of the available materials.

### I. Vitamin A and Carotene

1. Methods of determination: Concerning the determination of plant carotene contents, Tai Chung-kuang and Li Chien-hsing(1) reported that by using paper chromatography method and using petroleum ether as a solution, the recovery rate is above 90 percent, only a small amount of solution is needed; and the procedure is very simple. Tan T'ao-sheng and others(2) used propyl ketone-petroleum ether solution for the isolation of carotene. He also combined the Quackenbush's and Wall and Kelley's chromometry methods.

In the determination of Vitamin A, Ch'en jen-chun and others(3) have done some comparative studies about the vivo growth method and the method of determining Vitamin A contents in the blood or liver. Under their research condition (according our pharmaceutical rule it is allowed to prolong the time of Vitamin A deficiency in animal, to use chicken protein and vegetable oil instead of calcium protein in the

feed and add Vitamin E in the standard Vitamin A fat solution), the body weight of the Vitamin A deficient rat will increase when it is given 1-12 microgram of Vitamin A. The body weight increase is a straight line in relation to the total amount of Vitamin A intake. The increase of serum and liver Vitamin A content in the Vitamin A deficient young rat will be a straight line in relation to the total amount of the dietary Vitamin A when 1-10 microgram of Vitamin A is given. The increase also has a straight line relationship with Vitamin A contents in the liver and blood. The researchers considered that the vivo growth method is more reliable. Yang Tze-t'ien and Li Heng-chai (4) have done some preliminary studies on the determination of Vitamin A content by using the chromometry of Carr-Price reaction to find out the relationship between Vitamin A contents and light absorption. Thus the difficulty of using pure Vitamin A as an index is overcome. The researchers also found out that the bright red color reaction from Vitamin A and SbCl<sub>3</sub> remains stable for 2-5 minutes. The least examination amount needs 5-10 international unit/ml.

As for the isolation and concentration of carotene, Tai Chung-k'uang and others (5) have done some comparative studies. The conclusion was that the best results were obtained by filtering ethyl-ether.

2. Human absorption of carotene from vegetables: Li Chai-yin and others (6) have reported that the average rate of absorption was 20-40 percent from spinach, carrot, potatoes, dehydrated vegetable oil and dehydrated carrots.

3. Studies concerning night blindness and blood complement titer: Ch'en Jen-chun and others (7) have done some study on the mechanism of "mao-yun-hsiang" alkaline in treating night blindness. They observed Vitamin A contents in the blood and liver tissues during various Vitamin A nutritional conditions of rabbits after stimulating the nerve by injecting this alkaline or adrenalin. Mr. Ch'en and others considered that the therapeutic action of this alkaline may be due to the release of liver Vitamin A into the blood through nerve fiber. However, this is not a basic treatment.

Tan T'c-sheng and others (2) have analysed carotene contents in plants or drugs used for the treatment of night blindness. They discovered most of them contain only a small amount of carotene, and 13 kind of these drugs or

plants almost contain no carotene at all. Mr. Tan and others proposed to continue further research on its therapeutic mechanism.

Ku Chin-fan and others(8) have studied the Vitamin A, C contents in plasma in relation to the serum complement titer. The result was that the complement titer is persistent within a certain limit and does not change when the vitamin content changes.

## II. Vitamin D

1. Chemistry: Wang Yu(9) and others, while using potassium permanganate in ethyl alcohol or propyl keton for the purpose of oxidizing the Vitamin D<sub>2</sub> (in neutral or weak alkaline condition), obtained a new product. Its structure is 7,8 di-hydroxyl-7,8 dihydrogen Vitamin D<sub>2</sub>.

2. Bioassays: The Steenbock's diet is generally used to feed the rats to produce rickets for the purpose of Vitamin D bioassays. Chang Ch'ang-yin and Li Yu-jui used propyl ketone treated grains and bean-curd as the diet, which is better than the original diet for the rat to produce rickets. It is cheaper, easier to manage, and the protein supplied also has complementary value.

3. Increase the anti-ricketic effects of vegetable oil: In the search for a cheaper and simpler method to increase the anti-ricketic vitamins in diet, Chang Ch'ang-yin, Li Yu-jui and Ma Ching-ta'ng(10) have conducted some studies on increasing the anti-ricketic effects by exposing the rice-husk oil to sun-light. They reported that there is an increase of anti-ricketic effect. This study is very valuable for practical use.

## III. Thiamine

Shen Chi-ch'un and others(11) have studied the thiamine effects on internal reflexes. The results showed that intravenous injection of a large amount (80-300mg/Kg) of thiamine into dog or cat will cause a decrease of blood pressure, asphyxia and death. This action is due to central nerve system, because thiamine (2-100mg) stimulates the chemical receptor organ of the small intestine, posterial extremities, cervical artery and veins, and the aorta. Owing to the fact that reflex will cause increase of respiration and blood pressure, more than 50 percent of the ex-



periments showed that internal reflex is increased by acetyl-choline. Li JO-han(12) has reported that the thiamine has no influence in the process of tryptophan's change into arthranilic acid in the liver of rat.

Chang Hua-cheng and others reported a thiamine deficiency phenomenon in the non-thiamine deficient patient who suffered from heart failure. This phenomenon has no relation to the gastro-intestinal absorption ability of thiamine.

#### IV. Riboflavin

1. Determination methods: Wan Feng and Wang Shen-ch'uan(13) have done some preliminary riboflavin analysis by using the fluorescence quantitative analysis method. The result showed that the prepared products have no difference whether they were isolated by boiled water or not, the result will be much better if they are isolated by the high pressure method. The best results were obtained by precipitating impurities at pH 6.8 and by using NaOH for regulating PH. Chao Ching-hung and others(14) have improved the riboflavin analysis method. They used 0.01N KOH solution to increase the solubility of riboflavin, then neutralize it with 0.1N HCl so that the riboflavin will not dissolve within 3 hours. The effective range of analysis may increase from  $10^{-5}$  -  $10^{-4}$  to  $10^{-5}$  -  $10^{-2}$ .

2. Influence to metabolism and growth: Wang Wei-chou and others(15) reported the influence of riboflavin deficiency to rat's utilization of protein. During the riboflavin deficiency stage, the rat showed loss of appetite and slow down in weight gain. But when the amount of food intake is equal, the nitrogen balance and liver nitrogen storage of the riboflavin deficient group shows no difference from that of the control group except that urinary ammonia nitrogen is slightly decreased. Wang Chen-fa and Ch'en Ch'un-ming(16) reported the relation between riboflavin deficiency and the regeneration of protein in rat's liver, muscle and plasma. During the deficiency period, the composition ability of muscle protein is completely lost, and the regeneration of liver protein and plasma protein is also slow. Fang Chung-yu and Wang Ying-lai(17) have pointed out the close relationship between anthranilic acid hydroxylase and riboflavin.

Ch'en Hsueh-ts'un and others(18) have done one year's

observation of the growth of 87 middle school students, ranging from 11 to 15 years old. They discovered that there is a marked increase of the rate of growth when they are given 1mg of riboflavin and 1gm of calcium daily. There is a moderate increase of the rate of growth when they are given only 1gm of calcium daily; there has no remarkable influence when only 1mg of riboflavin is given as a supplement.

3. Biological composition: In searching for a cheaper and easily obtainable sources of riboflavin, Liu Pei-nan and Chen Wen-wei(19-22) have conducted some studies on the synthetic bacteria of riboflavin, that is, *eremothecium ashbyii* in riboflavin. They found most of the riboflavin was produced when *eremothecium ashbyii* is grown in a medium made of the millet, best-curd refuse and gluten. Every gram of the above will gain respectively 3, 4.2-6, and 4.5-6.0mg. The latter two are cheaper, easily obtainable and also simple to manage, and the products may be used directly.(20) Mr. Liu and others also conducted some study on the stimulating effect of myo-alcohol to the formation of riboflavin. The results showed that the myo-alcohol increases the utilization of glucose and nitrogen by the synthetic bacteria of riboflavin and that it may increase the respiration and growth and decrease the aging autolysis phenomena.(21,22)

#### V. Nicotinic Acid and Vitamin B6

At present, we already know that tryptophan will produce nicotinic acid in vivo experiments. The first step was metabolized by peroxylase of tryptophan. Lin Jo-han and Wang Ying-lai(23) have reported that the existence of this enzyme is closely related to Vitamin B6. Wang Ying-lai and others(17,24) reported that the nicotinic contents in rat's liver will decrease when Vitamin B6 deficiency occurred. They also studied that during the formation of nicotinic acid, the anthranilic acid changes to 3-hydroxy-anthranilic acid(17) (this hydroxylase is related to riboflavin).

#### VI. Vitamin B12

At present we all know, that the *E. Coli* can be used as a biological assay for Vitamin B12. Kao Chun-te and Chiao Shang-chih(25) reported that they used *E. Coli* 44110-1 for determining Vitamin B12 contents. This method

may be used without disturbance by histidine when Vitamin B<sub>12</sub> content ranges from 0.05 to 0.5mgm/ml. There is a slight influence of arginine, but it is very sensitive to proline. Wang Shu-chin and Shen Chih-ping<sup>(25)</sup> used carbo-activatus to absorb Vitamin B<sub>12</sub> and proline from the sample, and then washed off proline with PH 4.5 sodium acetic acid solution. The next step is to use 65 percent hot ethyl-alcohol to wash down Vitamin B<sub>12</sub>, then culture it with E. Coli 44110, and finally use its turbidity for the determination of Vitamin B<sub>12</sub>.

Recently, Vitamin B<sub>12</sub> was used in two cases for treating Leiner's disease<sup>(27)</sup>. Only a small dose gives excellent results (single dose is 0.0015mgm, generally 30-60mgm IM daily). This is worthwhile for further study.

## VII. Vitamin C

### 1. Determination methods:

(1) The method of determining food Vitamin C contents: Kuo Chen-ts'ai and Chang Ch'ang-yin<sup>(28)</sup> have pointed out when the 2,6 dichlorol phenol dropping method is used, the dehydrogen-antiscorbutic acid in the food should not be overlooked. The latter has the antiscorbutic action. Besides, they have determined the average anti-scorbutic acid content in the ordinary vegetables grown in Peiping; it is about 30 percent of the total Vitamin C amount. Fang Yun-chung and others pointed out that we have to pay attention to 2,4 di-nitrobenzol which may disturb 2-ketone glutamic acid in the sample. This substance has no antiscorbutic action but it will produce reaction with 2,4 di-nitrobenzol.

According to available literature, paper chromatography may be used to dissociate Vitamin C, but the amount obtained is rather low. Kuo Chen-Ts'ai and Ch'en Pen-mou<sup>(29)</sup> have proved this method and raised the recovery rate to more than 98 percent. Mr Kuo and Mr. Ch'en stated that the Vitamin C will be oxidized after paper chromatography. Accordingly, they used O<sub>2</sub> to expel the oxygen from the solution (water, 30% acetic acid, butyl alcohol), add certain amount H<sub>2</sub>S, washed off the anti-scorbutic acid after the chromatography, then added 2,6 di-chlore phenol preparation and then compared it by chromometer. H<sub>2</sub>S could not only protect antiscorbutic acid in the chromatography room, but also reduce part of the dehydrogenetic antiscorbutic acid. Kuan Lien<sup>(30)</sup> used the above method to obtain the antiscorbutic acid then reacted in tincture iodine solution.

He used the discoloration of the solution, caused by the reduction of iodine, to determine the antiscorbutic acid content. This color reaction is very sensitive, stable and simple.

Kuo Chen-ts'ai and others (31) have suggested the use of iodine instead of bromine as an oxidizing solution, and the use of solid sulpho-urea to remove the extra iodine. This method is simpler, safer and faster. Su Chen-chih and Ku Yuan-hsi (32) have used antiscorbutic oxidase instead of carbo activatum for the oxidation of antiscorbutic acid; they also added oxygen at PH 1.7, 37°C to destroy the disturbing substance such as the reduced ketone body. This is a simple method, but very specific.

Peng Hsien-sheng (33) has done some comparative studies on the accuracy of 2,6 dichloro phenol method, Laper and Vlademirov's electric light chrometer method and Stronsker's paper-chromatography method. They have also pointed out these good and weak points of these three methods and considered the second method is the best one.

(2) Determination of the blood and urinary Vitamin C contents: Schaffert and Kingsley have simplified the methods of Roe and Kuether for determining the blood antiscorbutic acid content. They boiled 5-10 minutes instead of keeping the temperature at 37°C for 3 hours as the original method. But the standard tube often becomes cloudy. Fang Chi-nan (34) and others have pointed out that the cloudiness was related to the incubating condition. They also considered that the original method or keeping the temperature under 56°C for one hour is more accurate. Miao-Chien and others (35) used the paper chromatography method of Kuo Chen-ts'ai for the determination of the antiscorbutic acid content in normal individuals. The result showed that there was no 2-ketone glutamic acid disturbing substance in the blood. According to this, Mr. Fang and others considered all the above mentioned methods could be used.

Ch'en Pan-mou and others (36) compared their own paper chromatography method with the method in which methyl aldehyde is used to extract the urinary antiscorbutic acid. They also pointed out the methyl aldehyde could not completely extract the urinary antiscorbutic acid. The most accurate and simple way is to use paper chromatography for determining the total amount of urinary antiscorbutic acid.

2. The influence of metabolism: In antiscorbutic acid deficient guinea pigs, the liver glycogen content was extremely low and the blood sugar level was higher than other normal animals. After giving them a large amount of glucose, the blood sugar increases in the same way as in normal animals; their liver glycogen content also increases markedly, but it is still lower than that of the normal group. According to this result and other experiments, the researchers proved that during the antiscorbutic acid deficient stage, the liver glycogenesis and glycogenolysis action in guinea pig is lower than in normal animals, but this has no effect to the liver phosphorylase activity (Ch'en Shuang-chi).<sup>(37)</sup> The adrenal glycocone solution from the blood of Cushing's syndrome patients has an inhibitory action to the glycogenesis in the adrenalectomized mice, but Vitamin C has the anti-action.<sup>(38)</sup>

Pan Kuang-hsi and others have reported that there is a relationship between the blood systino-glucoside and Vitamin C content in people who live 2250 feet above sealevel, but there is no such relation in people who live 3300 feet above sealevel. Mr. Pan and his associates have examined the natives and immigrants who lived 4200 feet above sealevel and the result showed no such relationship. Besides, as already described previously, the blood complement titer will not change when blood Vitamin A, C, contents change.

3. Biological composition: As we already know in animal experiments, the L-antiscorbutic acid was composed from the glucose through L-glucuronic acid- $\gamma$ -lactone or L-galacturonic acid- $\gamma$ -lactone. Chang Yu-tuan and Tung Lin<sup>(39)</sup> have proved that the enzyme which stimulates the above lactone oxidize to become antiscorbutic acid is distributed in the mitochondria of rat's liver cell. They also pointed out that this enzyme is closely related to riboflavin.<sup>(40)</sup>

#### VIII. Vitamin P

Su Ch'eng-chih and Wang Ch'eng-chi<sup>(41)</sup> have proposed a method by using "hua-ching-shu" reaction for the determination of citrin. This is a very specific method, the least examining dose is 0.025 mgm/ml, and the collection rate is 97.8-100.3 percent. Besides, the researchers have reported a method by using 50 percent of ethyl-alcohol for the isolation of citrin and pure citrin crystals. They also reported the effects of citrin in preventing frostbite.<sup>(42)</sup>

### IX. Vitamin Preparations

Kuo Feng-wen and others<sup>(43)</sup> have reported the industrial production of thiamine. The materials are easily obtainable, and no special equipment is needed. During the great leap-forward stage, success were reported in the experimental preparation of Vitamins A, D<sub>2</sub> and D<sub>3</sub> in crystal form. The preparation of crystal Vitamin A does not need the complicated paper chromatography method.<sup>(44)</sup> They also succeeded in preparing Vitamin B<sub>12</sub> from the waste solution of aureomycin.<sup>(45)</sup>

Generally speaking, we have done some work in the field of biochemistry of vitamin since the liberation, but not broad enough, not balanced and thorough enough. In the future, we should study more about the effects of the metabolism of vitamin in human body, pay more attention to the relationship between Vitamin A and the age, sex occupation, physiologic and pathological condition of different people, and also the metabolic problem of vitamin itself. By doing this we may raise the theoretically study of vitamin to a higher level, and could solve more practical problems.

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## BIOCHEMISTRY OF MICROORGANISMS

Following is a translation of an article entitled "Wei-sheng-wu ti sheng-wu-hua-hsueh" (English version above) by Liu P'ei-nan in Ch'ing-chu Chien-kue Shih-chou-nien I-hsueh K'o-hsueh Ch'eng-chiu Lun-wen-chi (Collected Essays on the Achievements of Medical Science in Celebration of the Tenth Founding Anniversary of Nation), Vol 1, compiled by the Ministry of Public Health, Peiping, pp 150-153 1959.

The study of biology and epidemiology of microorganism was started rather early in our country, but little work has been done in biochemical studies on microorganism except the preparation and assays of serum vaccine. Since the liberation of the whole country, owing to the encouragement and support of the Communist Party and the government, the scientists have realized the importance of the biochemistry of microorganism to the prevention and treatment of disease, so that recently, they have started the biochemical studies of microorganism. Most work was done concerning the production and effects of antibiotic combinations, and also some reports concerning the biochemistry of pathologic organism were prepared.

1. Biochemistry of Bacteria: As far as the bacterial biochemistry is concerned, Chang K'uan-hou and Yu Yung-Ch'uan have proved that there is substance which inhibits the V-factor of bacillus influenzae in blood stream (some people have suspected that this is coenzyme 1). Under 7000-10000 and heating for 5-10 minutes, this inhibitory substance will be destroyed and the V-factor will be released. By putting a certain amount of Coenzyme 1 and chloride hemoglobin in the liver broth as a source of V-factor and X-factor, this may take the place of chocolate medium. The highest amount of oxygen consumed by bacillus influenzae is in the chocolate liver broth; the next is in the liver broth which contains Coenzyme 1 and chloride hemoglobin; the weakest respiration is in the liver broth which contains no such factors.(1)

Wang Ta-sheng and Meng Wei-lien<sup>(2)</sup> have developed a bacterial mill which needs only a short period of time and has a very high efficiency. The oxidase activity of the products, such as succinic acid is very high. Many of the Coenzymes were not lost, so they could oxidize the succinic acid, L-lactic acid, methyllic acid, L-glutamic acid, L-aspartic acid and ethylic alcohol. The E. Coli bacterial cell broth obtained by using the above mentioned bacterial mill could be divided into 9 portions by paper electrophoresis. They found out that 0-50 percent of the protein precipitated from ammonia sulphas moves very fast in the electrophoresis. When the ammonia sulphas concentration increases, the precipitated protein will move slower.<sup>(3)</sup>

In the studies of the mechanism of protein composition, they discovered that both E. Coli and lactobacillus acidophilus could utilize N-phosphoric acid-DL-benzedrine acid and methylis. They found that the utilization efficiency was lower than that of benzedrine. Further experiments have proved that E. Coli, yeast and lactobacillus all could dissociate the activity of N-phosphoric acid-DL-ammonia benzedrines.<sup>(4)</sup>

In the area of antibiotic effects on the bacteria, Wang Yu and his associates<sup>(5)</sup> have observed Chloromycetin could inhibits the respiration of staphylococcus aureus; about 0.04-0.08 mgm-molecules of chloromycetin in the culture broth could markedly decrease the respiration of this bacteria. The guinea pig serum seems to be able to protect this bacteria from chloromycetin effect. The E. Coli has a stronger resistance to this antibiotic. In the phosphate buffer solution, even 16 mgm-molecules of chloromycetin still couldn't inhibits the respiration of this bacterium. The succinic acid oxidase system was very sensitive to chloromycetin. The L-amino acid oxidase, cytochrome oxidase and succinic acid dehydrogenase all could be inhibited by this antibiotic. The chloromycetin in low concentration could stimulate carbonxylase, but will inhibit it in high concentration.<sup>(6)</sup> The aureomycin effect on the respiration of E. Coli is related to the nitrogen source of the culture medium. The phosphoric acid buffer solution, which only contains glucose, still has no remarkable effect on this bacterium even 100 mgm/ml of aureomycin is added. But if any nitrogen source is added, then the salution which contains 2.5 mgm/ml of aureomycin could inhibits the growth of E. Coli. Mr. Wang and his associates considered that this inhibitory action is based on the metabolic process of

the carbon and nitrogen combination. (7) With the E. Coli liquid medium, in which amino acid is formed by utilizing the inorganic nitrogen, they have used the method of fractionation to examine the aspartic acid, glutamic acid, alanine, 4-threonine, valine and isoleucine. Among these six, the glutamic acid decreases according to the length of culture time, while 4-threonine will increase if the culture time is prolonged. If terramycin (1 mgm/ml) is added to this medium, 4-threonine will decrease markedly, but the valine will increase markedly. The decrease of 4-threonine may be related to the decrease of the glutamic acid decarboxylase activity, but this action is not clear during the experiments about terramycin's inhibitory action on this enzyme.

In the medium of streptomycin dependent strain E. Coli, as opposite to the antibiotic action, the E. Coli grows abnormally if the medium contains no streptomycin, and the oxidation activity of glucose, analin, succinic acid, malic acid, lactic acid. Will decrease markedly. The protein contents in the bacterial body also will decrease, but their nucleic acid shows increase. The decrease of metabolic oxidation is considered related to the influence of protein composition. (8,9)

In the area of antimicrobial action of Chinese drugs, members of the biochemistry teaching and research group of the Sze-Ch'uan Medical College have done a series of studies in this field. They observed that the "Ch'uan-huang-lien" compound (10 kinds) could inhibit the respiration of staphylococcus aureus, and also inhibits the dicarbonic oxidase of the pyruvic acid,  $\alpha$ -keton-glutaric acid, and the dehydrogenation of glucose, lactic acid, succinic acid and malic acid. The nicotinic acid, Vitamin B<sub>6</sub>, aminobenzoic acid, nucleic acid and nucleoglucuronic acid all could resist the bacterial inhibitory action of 11 kinds of Chinese drugs, such as "huang-lien-shu", "huang-pe", and "huang-chin". Besides, the respiration of the bacteria, and their ability to oxidize the pyruvic acid and  $\alpha$ -keton-glutaric acid could be recovered to a certain degree.

2. Biochemistry of antimicrobial bacteria: In the area of antimicrobial bacteria (including streptomycetes and mildews), they have conducted many experiments on the fermentation condition of antibiotics and the theoretical researches, so that industrial production could be achieved. Chang Wei-sheng and others have used the cotton-seed cake

instead of the corn-broth in the *Penicillium Chrysogenum* medium, (10,11) and starch and corn-starch instead of the above mentioned for lactose fermentation. (12,13) Besides, they have prepared reports concerning the preparation of streptomycin, (14) aureomycin, (15) and terramycin. (16)

In the study of antimicrobial bacteria physiology, most of the work was done on aureomycin. They have proved that by using an adequate inoculated medium, the amount of aureomycin can be increased by 7-8 times. The aureomycin nitrogen utilization is selective. The phosphate could inhibit the aureomycin composition and could increase the activity of the glucose oxidase system. When an adequate carbon source and an adequate medium are selected, the phosphate could also increase the production of aureomycin. The effective mechanism of the phosphate is based upon the fact that the phosphate inhibits the activity of 6-phosphoglucohydrolase and changes its metabolism to the direction of Embden-Meyerhof-Parnas. (17-21) Besides, in regard to the metabolic changes during the process of aureomycin fermentation, they have done some observation on the change of the acid and alkaline, utilization of glucose, nitrogen metabolism, the concentration changes of ammonia and organic acid, and the respiration of myces. (22,23) They also did some observation on the oxidation and reduction changes in the aureomycin medium. (24) They studied the iron ion contents of this medium in connection with the aureomycin production, and proved that the iron ion could combine with aureomycin, (25) acidify the fermented solution, and then increase the aureomycin titer. (26) After the medium is treated with ultraviolet ray, and the two low productive myces cultures are mixed together, the aureomycin products will increase. (27,28)

In connection with the physiology of streptococcus cinereus, they have studied the nutrition of the streptococcus cinereus, and its growth in various sugars in connection with streptomycin production. In regard to the influence of sodium ion on these bacteria's growth and the production of streptomycin, they found out an optimal concentration. (29,30)

3. Other studies: In the study of microorganism technique and application, they have established a microorganism determination method which method is very simple and sensitive. Aside from the analyses of amino acid and vitamin contents in ordinary food, they have prepared some reports

concerning the improvement of the culture medium, such as the use of *lenconostoc mesenteroides* P-60 to determine lysine, (31) the use of *lactobacillus arabinosus* to determine the food amino acid, (32,33) the use of *E. Coli* 44110-1 to determine the proline, (34) and the use of *E. Coli* to determine Vitamin B<sub>12</sub> in food. (35)

Besides, in comparing the soybean protein acid hydrolytes with enzyme hydrolytes (when used as the culture medium for typhoid vaccine) they found that the former decomposed completely, the tryptophan was destroyed, and the bacteria growth was rather poor, but it will not produce protein shock and sensitive reaction in guinea pig. They also found that the latter decomposed incompletely, the bacterial growth was good, but will cause shock and sensitive reaction. If tryptophan is added into the acid hydrolyte and then the ventilation method is applied, the growth of this bacterium (36,37) will be stimulated. In the study of dysentery bacillus culture medium, they have studied the influence of various bile salts on the growth of this bacterium. Some experiments were also done on *E. Coli* and typhoid bacillus. They prepared an excellent medium by using the salt solution extracted from the cow's heart. (38,39) They also prepared soy bean protein and casein albumin hydrolytes. They found that procedure is simple, the time required is short and no antiseptics (40,41) are needed. The protease from soil is very active; it could digest the soybean protein, and could be prepared as an excellent protein peptone. (42,43)

Fang Hsin-fang and others have conducted studies on the activity of several protease and the carbon and nitrogen sources of the medium in relation to the production of this enzyme. (44,45,46)

Owing to the nutritional necessity of the riboflavin composition, Ch'en Wen-wei and others have studied the morphological change of *eromothecium ashbyi* during its development stage, (47) the inositol influence on this bacterium's metabolism and its composition of riboflavin. They proved that inositol has a stimulating action on the development of this bacterium, and also delayed its autolysis. (48) Besides, they also studied the amount of optimal inositol, which could increase the production of riboflavin. and in the experiments of single amino acid medium, while comparing with other amino acid, they found that glucosacetylamide and aspartic-acetylamide could produce more

riboflavin. Since the liberation, we could see from above achievements, that the biochemical research work has been mainly directed at meeting the country's needs. But there are many areas in which studies still in an early stage. Further efforts should be directed at the study of pathogenic bacteria's metabolism, the efficacy of Chinese drugs and antibiotics, and the biochemistry of the toxin in relation to the host.

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## ACHIEVEMENTS IN THE BIOCHEMISTRY OF PARASITES

Following is a translation of an article entitled "Chi-sheng-chung sheng-wu hua-hsueh ti ch'eng-chiu" (English version above) by Huang Tso-yueh of the Parasitic Disease Research Institute of the Chinese Medical Science Academy, in Ch'ing-chu Chien-kuo Shih-chou-nien I-hsueh K'c-hsueh Ch'eng-chiu Lun-wen-chi (Collected Essays on the Achievements of Medical Science in Celebration of the Tenth Founding Anniversary of the Nation), Vol 1, compiled by the Ministry of Public Health, Peiping, 1959, pp 153-156.

The biochemistry of parasites is still in a stage of infancy. In the early part of the 20th century, some work was done in this field, but it didn't receive much attention. In the past 15 years, owing to the excellent achievements in biochemistry of microorganism, the biochemical research workers of the entire world have been interested in the biochemistry of parasites, and they are continuing further studies in this field.

It was an untouched field in our country. Since the liberation, owing to the close attention and support given by the Chinese Communist Party and our government, the parasites research work has build up a good fundation, and it is on the way to forther development. Because the flukes are very harmful to people's health, especially to our workers' health, most of our work were done in the area of schistosomiasis.

### I. The Chemical Composition of Parasites

The basic information for parasite biochemistry research are the weight of the parasites and their organic and inorganic chemical compositions. T'ao I-hsun and Ma Li-jen have reported the percentage of the dry weight of several flukes: *Schistosoma Japonica* male, 20.4 percent, female 24.1 percent; *paragonimus* 14.9 percent; *fasciola hepatica*, 20.3 percent; and *fasciola gigantica*, 15.8 percent.

(1) The body weight of each kind of fluke is influenced by several factors, such as the difference between infected hosts, duration of infection and other factors. Mei Kuang-yao has determined the average body weight of male and female *Schistosoma* after 36, 56 and 66 days of infection in mice. The results, in time sequence, were 30, 92 and 95 mgm in female worm; 83, 130 and 157 mgm in male worm. (2) As the body weight of the worm increases, its nitrogen and phosphorus contents also increase. But as for as the body weight percentage is concerned, there is no significant change. The average nitrogen content in the female worms is 8.7-10.1 percent; phosphorus, 1.00-1.41 percent. As for the male worms, the average nitrogen content is 7.2-7.5 percent, and phosphorus content is 0.69-0.73 percent. In each pair of *Schistosoma Japonica* which grow in guinea pig, the total nitrogen content is 24-31 mgm after 50-75 days of inoculation.

The glycogen in parasites is widely distributed. According to the result from determination, the glycogen content in female *Schistosoma Japonica* is 0.5-1.0 percent of its wet body weight and in male *Schistosoma Japonica* is 1.9-2.9 percent; the glycogen content in *paragonimus* is 3.57-4.68 percent of its wet body weight; that in *Fasciola hepatica* is 2.17-2.91 percent of its wet body weight; and that in *Fasciola gigantica* is 4.08-5.44 percent. (1)

In the infected rabbits which had been treated with tartaremitics for three days (the total dose was 21 mgm/kg of body weight), the autopsy findings within 30 minutes showed marked decrease of glycogen in the *Schistosoma*. It decreased to about 1/3 of the normal amount, but most of the glycogen could recover after 12 hours. Ch'en Te-hui and Chu Chi considered that glycogen was the source of energy. After the flukes went into the liver, the male and female flukes separated from each other and then returned to the original location. These changes are closely related to the contents of glycogen.

By using paper chromatography in the study of hydrolytic polysaccharides obtained from the male *Schistosoma* and the ovum, they found that the polysaccharides in ovum are composed of glucose, galactose, mannose, xylose and other monosaccharides, but the properties of these monosaccharides are not fully understood yet. The polysaccharides in male *Schistosoma* contain only glucose and galactose. (3)

The protein content of schistosoma may be found through the amount of nitrogen multiplied by 6.25. The protein content in a male worm is 45-47 percent of its dry body weight, and that in a female worms in 55-64 percent of its dry body weight. The electrophoretic patterns of the protein in male, female and ovum are entirely different. The male worm's protein showed 3 peaks, the female worm's protein has 2 peaks and ovum has 3 peaks. This clearly demonstrated the differences in their composition. In the study of protein composition in Schistosoma, they have used the method of electrophoresis but it couldn't isolate each component part of the protein. The protein in the paper electrophoretic pattern only showed a band-like distribution located between  $\alpha$ - $\beta$  globulin in human serum. There is only a small amount of fat protein and sugar protein in Schistosoma. (3)

The chemical composition of fasciolopsis buski as follows: water  $73.3 \pm 0.6$  percent, solid  $26.3 \pm 0.62$  percent. The solid consists of the following: fat  $6.8 \pm 0.4$  percent, ashes  $3.89 \pm 0.03$  percent, protein 44.7 percent, and glycogen 40.3 percent. (4)

The hemoglobin of fasciola hepatica may be decomposed into co-enzyme and protein. They believed that the co-enzyme in the hemoglobin of fasciola hepatica may be similar to the co-enzyme in cow's hemoglobin. Meanwhile, chloride hemoglobin could be obtained from the hemoglobin of fasciola hepatica. Its hemoglobin has a stronger combining power with oxygen than ordinary blood. Hence this hemoglobin may be similar to the cow's hemoglobin, but they are not the same compound. (5)

Acetyl-cholinesterase not only could be found in the vertebrates, but also could be found in the low class animals. At present, its physiological ability and mechanism are still not quite clear, but continued research work in this field is still going on. In foreign countries, it has already been proved that this enzyme could also be found in Schistosoma mansoni, and that the enzyme's activities in male and female worms are almost the same. But according to the study of Shen and his associates, it is not so in Schistosoma japonica. When the ground substance of acetyl-choline was put in an appropriate solution (concentration  $5 \times 10^{-3}$  -  $1 \times 10^{-2} M$ ), then the activity of acetyl-cholinesterase in male worms is 3 times stronger than in female worms. One mgm of dry worm could hydrolysis 126 mgm of

acetylcholine per hour. Atropin sulphate could increase the activity of this enzyme, while salicylic acid could inhibit its activity. As the tartar emetic solution at a concentration of  $1 \times 10^{-3} M$  could only inhibit about 10 percent of this enzyme's activity, the use of tartar emetic for the treatment of schistosoma is not related to acetylcholinesterase.

Huang Ju-heng and others have used the Thunberg tube for the study of fasciolopsis buski. They have proved the existence of succinic acid dehydrogenase and glycerophosphate dehydrogenase in fasciolopsis buski. Besides, it also contains a considerable amount of acid phosphatase. (4) There are some reports concerning studies about fasciola hepatica. It is found that it contains cytochrome oxidase and amino acid oxidase but not in large amount. However, the acid and alkaline phosphatase content is very rich. They also proved that there are phosphoric acidase, succinic acid dehydrogenase and protease, but no urease in the body of fasciola hepatica. (5)

## II. The Metabolism of Parasites

In the study of biochemical metabolism of parasites, researches on sugar metabolism are more intensive. But our knowledge in this area is still limited. Generally, it is considered that the source of energy of Schistosoma mansoni is mainly from carbohydrate fermentation. Primary studies of carbohydrate metabolism in the culture medium of Schistosoma japonica showed that this worm's sugar fermentation is closely related to the composition of the medium. In the 0.066 M Bueding phosphate buffer solution medium (pH 7.7), the amount of glucose consumption and the amount of acid produced by this worm are very high, almost double those in the McNaughton medium (0.01 M phosphate buffer solution, pH 7.4). After being in the Bueding medium for 3-4 hours, the worm's activity becomes very weak and its  $QO_2$  (ml/Nitrogen mgm/per hour) also decreases gradually. The concentration of phosphate might be an important factor, and its R.Q. (respiration quotient) is close to 1.

Riboflavin and antiscorbatic acid have a very remarkable influence of this worm. The reason of this is that when the medium contains these two vitamins, then the worm's  $QO_2$  increases 25 percent, and the respiration rate per hour remains even during the cultivation period. The activity of the worm becomes very strong after cultivation.

Cystein, Ca-pantothonate and adenosine-triphosphate also have the same action. Owing to the fact that Ca-pantothonate, adenosine and cystein are the constituent parts of co-enzyme A, it is suspected that when cultured in a medium which contains these three compounds, the increased activity of this worm might have a definite relation with co-enzyme A.

From the literature, Vitamin K<sub>3</sub> has a strong inhibitory effect on the carbohydrate fermentation of schistosoma mansoni, but it has no remarkable influence on respiration. In Schistosoma Japonica, the condition is not so. Vitamin K<sub>3</sub> (final concentration 0.00015 M) has a strong stimulating action on this worm's respiration during the first hour of cultivation, the QO<sub>2</sub> being 280 percent higher than the non-Vitamin K<sub>3</sub> control group. It will decrease gradually till the third hour of cultivation when it is only about 10 percent of the control group. This change is very great. The glucose consumed and the acid produced also decreases markedly, and the R.Q. is 0.80. The relationship between the rate of decrease of fermentation and the fluctuation of QO<sub>2</sub> has not yet been clarified.

They also did some study on the normal sugar metabolism of Schistosoma Japonica and the influences of drugs. (7) Tartar-emetic and iodo-acetic acid could inhibit the sugar fermentation in this worm, but its activity seems to have no close relationship to the intensity of fermentation.

There are also some reports concerning the gas metabolism in fasciolopsis. (4) The QO<sub>2</sub> in a complete fasciolopsis is related to the partial pressure of oxygen, the pressure of pure oxygen being twice as high as that of oxygen in the air. The sugar fermentation rate is also very high in fasciolopsis.

Gas metabolism in Fascilo hepatica has been reported by Shao Chi-chih, Lin Kuo-kao and Wu Kuang. (8) The QO<sub>2</sub> will change while the partial pressure of oxygen changes; this is different from the results reported by foreign countries.

### III. The Culture and Nutrition of Parasites

If anyone could develop an ideal medium in which the parasites may still live and carry on their normal physiologic activity, and their lava may grow into adult hood, it will be a great help to the studies of the physiology,

biochemistry and pharmacology of parasites. In fact, the study of cultivating parasites in vitro has been systematically developed in our country since 1951. There have already been comprehensive reports on this subject. (9,10) According to the result of their research, it is discovered that the flukes showed excellent growth in the mixed medium of serum-tyrode and glucose solution, and that the best serum was obtained from ass and mule. If liver extract is added into the medium, it will be more helpful to keep the parasites alive. When less than 1,000 units of penicillin and streptomycin is put into the medium, there is no remarkable influence on the activity of schistosoma; nor do they have any toxic effect on the parasites. The vitamin have a certain influence on the life span of the flukes. In each milliliter of tyrode solution containing 100 mgm of Vitamin C the average life-span of schistosoma is about 5.95 days, longer than generally reported.

Some studies were already started on the amino acid influence to schistosoma Japonica in vitro (11). Among the 25 amino acids under experiment, 5 of them, namely, L-hydroxyproline, L-proline, L-cystine, DL-valine, DL-phenylalanine can prolong the life span of schistosoma. Generally, the male worm lives 2-3 times longer than the female worm. Besides, the concentration of amino acid is also related to the duration of life. In view of the reports in foreign literature, they thought that too little amino acid was used in the culture medium by foreign researchers. Perhaps this is why their result was rather poor as far as the life span is concern.

After a pair of schistosome are inoculated into the arterial chamber of rabbit's eye, the female and male parasite will soon separate from each other. The male worm could live several months long in the arterial chamber, but the female worm usually dies within a few days. (12)

#### IV. Summary

Although the parasite is a kind of organism whose existence depends mainly upon the host, yet it has its own physiologic action and biochemical metabolism characteristics. The parasites draw nutrition from the host and also secrete metabolic products into the host body. Both action will cause of parasitic disease in human body. A clarification of the biochemical characteristics will be very helpful to the understanding of the mechanism and treatment

of parasitic diseases. The research of parasite biochemistry not only is very important to the theory of comparative biochemistry, but also has a practical meaning in treating parasitic disease. In the last 30 years, the achievements in microorganism research has foreshadowed a bright future for the biochemistry of parasites. We believe that on the basis of the present achievements, the science of parasites will develop with a very high speed.



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## RESEARCH IN CLINICAL BIOCHEMISTRY

Following is a translation of an article entitled "Lin-ch'uang sheng-wu hua-hsueh ti yen-chiu" (English version above) by Wang Shih-chung, in Ch'ing-chu Chien-kuo Chih-chou-nien I-hsueh K'o-hsueh Ch'eng-chiu Lun-wen-chi (Collected Essays on the Achievements of Medical Science in Celebration of the Tenth Founding Anniversary of the Nation), Vol 1, compiled by the Ministry of Public Health, Peiping, 1959, pp 157-164.

Since the liberation of the whole country, there has been a great progress in the field of clinical biochemistry, which includes biochemical diagnosis, biochemistry of occupational disease, biochemical products and the improvement and the establishment of test methods in pathological metabolism. Following are the reports concerning these fields:

### I. Biochemical Diagnosis

The main work in this area was the application of modern biochemical methods and theories for the study of biochemical index changes under pathological conditions to help clinical diagnosis of diseases. When the researchers were determining the biochemical index among patients, they also determined the same index for the normal people. They have thus collected valuable material on the biochemical composition of the blood and urine of the people in our country.

Ma Yu-cheng<sup>(1)</sup> has determined the serum protein contents in normal individuals in our country. He discovered that the average serum protein value is 6.50 gm percent, of which 3.92 gm percent is albumin, 2.32 gm percent is globulin and 0.28 gm percent is fibrinogen. In globulin, 0.65 gm percent is  $\beta$ -globulin, 1.14 gm percent is  $\alpha_1$ -globulin, and 0.53 gm percent is  $\alpha_2$ -globulin. The A/G ratio is 1.73. Li Yun-sheng<sup>(2)</sup> and others have determined the serum protein contents among our college students. He discovered that the average serum protein content is 7.28 gm percent, which includes 4.08 gm percent of albumin and 3.20 gm percent of globulin. The A/G ratio is 1.30.

Several people(3-7) have used the filter paper electrophoresis method for the study of serum protein composition. They have studied normal people and various sick patients. Following is one of their reports: albumin, 57.0±2.3 percent;  $\alpha_1$ -globulin, 4.0±0.8 percent;  $\alpha_2$ -globulin, 9.3±0.9 percent;  $\beta$ -globulin, 14.7±1.3 percent;  $\gamma$ -globulin, 14.9±1.4 percent. Under various pathological circumstances, each portion of the serum protein will show remarkable change. For instance, during liver disease, nephritis, leukemia, pneumonia, rheumatic fever, rheumatic heart disease, Schistosomiasis, lupus erythematosus, multiple myeloma, etc. The protein content in the patient decreases markedly, but  $\gamma$ -globulin always increases.(3-7) Aside from above-mentioned changes, each disease also has a specific change. For instance, in liver cirrhosis patients,  $\beta$ -globulin will increase;  $\alpha_2$ -globulin will increase in patients with malignant tumor;  $\alpha_1$ -globulin will increase in patients with rheumatic heart disease. They also discovered a so-called "M-globulin" in patient with multiple myeloma.(3-7)

The average lipoprotein content of a normal individual in our country is as follows:  $\alpha$ -lipoprotein, 35 percent;  $\beta$ -lipoprotein, 65 percent. Total serum cholesterol contents is 165.2 mgm percent, total phospholipides average is 170.3 mgm percent. The cholesterol ratio between  $\alpha$ -lipoprotein and  $\beta$ -lipoprotein is 32:68, the phospholipides ratio in  $\alpha$  and  $\beta$  lipoprotein is 48:52; the ratio between serum cholesterol and phospholipides is 0.98.(8) In certain diseases, the serum lipoprotein and its composition will change markedly. For instance, in liver disease especially liver cirrhosis patients, serum  $\alpha$ -lipoprotein will decrease but  $\beta$ -lipoprotein usually increase markedly. In some severe liver cirrhotic patients, serum  $\alpha$ -lipoprotein almost disappears completely. Similar conditions are found in patients with arterial atherosclerosis. In patients with myocardium infarction, the serum cholesterol and  $\beta$ -lipoprotein cholesterol are higher than in normal individuals, but  $\alpha$ -lipoprotein cholesterol shows decrease. The serum cholesterol/phospholipides ratio may rise up to 1.07. The serum cholesterol also increase in patients with hypertensive arterial sclerosis, but lipoprotein cholesterol has no remarkable change.(8) All above studies have further proved that the determination of serum protein, lipoprotein and its composition are very helpful to clinical diagnosis.

There are many specific reactions of serum which are closely related to the chemical and physical properties of serum protein, such as (1) cephalin cholesterol floccula-

tion reaction, (2) thymol turbidity reaction, (3) thymol flocculation reaction, (4) zinc turbidity reaction, (5) copper turbidity reaction, (6) gold complement reaction. All these are very valuable to clinical diagnosis and differential diagnosis of various disease. For instance, (1) and (4) are helpful to the diagnosis of acute liver disease; (2,3) and (4) are helpful to the diagnosis of chronic liver disease; (6) is quite valuable to the differential diagnosis between obstructive jaundice and hepatocellular jaundice; (2,3) and (6) are helpful to the diagnosis of chronic hepatitis. Besides, these tests are also useful in the differential diagnosis between primary liver malignancy and secondary liver malignancy. Some times these tests also show positive reactions in the serum protein of non-liver disease patients, but generally it is a very weak positive reaction. (9-12)

The determination of serum glutamic acid-oxaloacetyl transaminase is very valuable to the differential diagnosis of the following diseases: (1) acute myocardium infarction and acute endocardium necrosis (markedly increased activity) in differentiation from angina pectoris (activity normal); (2) acute hepatic jaundice (markedly increased) activity in differentiation from obstructive jaundice (slightly increased activity); (3) brain hemorrhage (increased activity) and other cerebral vascular accident (normal activity). (13)

The activity of cholinesterase will decrease in the blood of acute hepatitis, portal cirrhosis, biliary cirrhosis patients and some other liver cancer patients; it also markedly decreases in hepatic coma patients. This enzyme is also useful in the differential diagnosis of obstructive jaundice and the hepatocellular jaundice; it is also helpful in determining the prognosis of chronic hepatitis, but the serum cholinesterase activity usually decreases in the patients with cachexic or pernicious anemia. (14)

The determination of serum amylase and lipase are also useful in the diagnosis of disease. For instance, in acute pancreatitis both enzymes will suddenly increase, especially serum amylase will increase markedly; this is very valuable in early diagnosis of acute pancreatitis. The serum amylase activity also increases in acute parotitis, but there is no change in the lipase activity. Besides, as the blood amylase increases, the urinary amylase activity also increases, so that the determination of urinary amylase activity has a diagnostic value. In patients with submandibular lymphadenitis, scarlet fever and measles, the

activity of amylase is within normal limits. (15,16)

The ammonium determination of blood has a great value in the diagnosis and prognosis of disease, especially in liver disease. The blood ammonium content in normal individuals in our country is 40-120 mgm percent, the average being 78 mgm percent. (17) The blood ammonium will increase in patients with liver disease, especially in those whose liver tissues are diffusely damaged. The ammonium will increase markedly when the patient is in hepatic coma. (19) So that, the main cause of nervous disturbances is due to the disturbance of ammonium metabolism in patients with liver disease. The determination of blood ammonium could reflect, to a certain degree, the condition of portal collateral circulation and liver tissue damages.

In patients with hepatic coma, the change of blood glutamic-acetylamide content is rather great, but it has no statistical value. Their blood glutamic acid content has no great difference in comparison with that of normal individuals.

The determination of blood iron contents is very valuable in the differential diagnosis between obstructive jaundice and hepatocellular jaundice. In obstructive jaundice patients the serum iron content is lower than in normal individual, but it will increase markedly in patients with acute infectious hepatitis. The serum iron content has no significant change in patients with chronic hepatitis. However, if the iron content increases markedly, then more attention has to be paid to their diagnosis and treatment. (20)

There are also many reports in the area of clinical biochemistry of endocrine (see the achievements in the field of endocrine). For instance, Chang Kuo-lin (21) has studied the urinary secretion of 17-ketosteroid and 17-ketone corticosteroid among various age groups in our country. He has discovered that the largest amount of 17-ketosteroid secretion is found in people about the age of 25 (male or female); for male, the average is 14.46 mgm/24hrs, for female the average is 12.05 mgm/hrs. Among the male, the largest amount of urinary 17-ketosteroid is found in men about the age of 30-40 (16.10 mgm/24hrs). Among the female the largest amount is found in women about the age of 20-30 (15.7 mgm/24hrs). Sun Chia-shou (22) has studied the urinary secretion of formadehyde-cortico-steroid

in our country. Clinically, the determination of urinary 17-keton-steroid is helpful in the diagnosis of primary or pituitary adrenol insufficiency (decrease of urinary 17-keton-steroid); adrenal syndrome (increase), and Etsenko-Chahing's syndrome (increase). Observation of the urinary 17-ketone steroid secretion after injection of ACTH is very helpful to the understanding of adrenal cortex function.<sup>(23)</sup> The urinary 17-ketone steroid decreases markedly in patients with Sheehan's syndrome and Addison's syndrome.<sup>(24)</sup> In patients with acute infectious hepatitis and liver cirrhosis, the urinary 17-ketone steroid content will be lower than in normal individuals, but their urinary phenolsteroid secretion will be higher than normal individuals.<sup>(25)</sup>

The determination of in the tissue fluid of chest cavity has been used in the differential diagnosis between T.B. and non-T.B. patients, but its value is still undetermined. Some researchers stated that if the sugar content is under 50 mgm percent then there is indication of T.B., but if the sugar content is over 80 mgm percent, then there is no indication of T.B.<sup>(26)</sup> But most researchers do not consider this conclusion significant.<sup>(27-30)</sup>

There are also reports concerning the cholesterol content in the tissue fluid of chest cavity. They have studied 27 cases and found the cholesterol content is between 22.5 and 207.0 mgm percent, the average being about 106.8 mgm percent.<sup>(31)</sup>

Li Yun-sheng and others<sup>(2)</sup> have done some survey among our college students, such as the determination of urea nitrogen, urea, muscle acid, muscle anhydride, total serum cholesterol, lipocholesterol, free cholesterol, serum calcium, and organic phosphate. Sun Chia-shou<sup>(32)</sup> has determined the plasma amino acid contents among our college students.

## II. Biochemistry of Occupational Disease

Many scientists have studied the problem of lead poisoning. They discovered the appearance of basophilic granules in the red blood cell and the so-called "stippling red blood cell" in the patient of lead poisoning.<sup>(33)</sup> The urinary lead and porphyrine secretion of such patients are higher than normal individuals. This is very important in the early diagnosis of lead poisoning.<sup>(34-38)</sup> Besides, some scientists have established a simple method for testing

urinary porphyrine--red ring test.(39) This method is also helpful in the early diagnosis of lead poisoning.

In patients with benzene poisoning, there is a persistent decrease of leukopenia and increase of lymphocytes; this is be very helpful in clinical diagnosis of benzene poisoning.(40-42) Some researchers have also studied the urinary secretion of inorganic phosphate and organic phosphate in benzene poisoning patients.(43)

Jen Tan-feng,(44) Chang Yu-kuei and Yu Pei-ch'uan(45) have studied the urinary mercury secretion in patients with mercury secretion in patients with mercury poisoning. They considered the urinary mercury amount cannot be used as an indication of the severity of mercury poisoning; it only tells that mercury has invaded the body.

Chou Te-lin, Tung Chi-yung(46) have studied the urinary and blood magnesium contents in patients with magnesium poisoning. Mr. Chou and others considered that the amount of blood and urinary Mg could not be used in clinical diagnosis; it may only be used to help the diagnosis.

Chang Hua-cheng and others(47) have done some studies on laborers working in high-temperature environments. They have observed changes of blood propio-keton acid, lactic acid and  $CO_2$  in these workers, and also observed their secretion of lactic acid and propio-keton acid. They found that there is no significant change such as increase of urinary or blood lactic acid and propio-ketone acid. The amount of  $CO_2$  is also normal. But when were working and sweating in a high temperature shap, then these three substances all markedly increase in the sweat. The researchers considered that the sweat not only has a body temperature regulation function, but also is very important to acid-base balance. Meng T'ien-cheng and others(48) have discovered that these workers' urinary nitrogen, sweat nitrogen, urinary dium and sweat sodium are much higher than ordinary people.

In 1958, the Chinese Medical Institute has organised a silicosis research team and studies were conducted among thousands of silicosis patients in Kiangsi province. In the area of biochemistry, they found that the compound of serum protein and hexose and the compound of serum protein and amino hexose were markedly increased in silicosis patients. Besides, the ptyalinase activity also increases in



silicosis patients. These reports are very useful in the early diagnosis of silicosis.

### III. Pathologic Metabolism

Wilson's disease is caused by the disturbance of copper metabolism. In Wilson's disease patients, the contents of copper in various tissues especially the liver and brain tissues<sup>(49)</sup> are all higher than in normal individuals. Besides, the urinary copper secretion also increases, but the feces and blood copper contents have no change or even decrease.<sup>(50)</sup>

The blood sugar of content leukemic patients is lower than normal individuals, and the decrease degree is proportional to the number of W.B.C. The glucose tolerance curve is also lower than that for normal individual. Because of the fact that the leukocytes contain glucose fermentation enzyme, blood sugar determination has to be done immediately after the blood is collected or an optimal amount of inhibitory agents (fluoride sodium) must be added, otherwise the blood sugar will decrease gradually and gives false results.<sup>(51)</sup>

Glossitis, perleche and scrotum dermatitis are closely related to the deficiency of nicotinic acid and riboflavin. If the scrotum dermatitis patients take 10 mgm of riboflavin orally, 50 percent of these patients will show symptomatic improvement on the 3rd day, and complete recovery on the 6th day for most of the cases. The scrotum dermatitis patients who only receive nicotinic acid without riboflavin, the symptom will be worse. Hence, scrotum dermatitis has been considered as a characteristic of riboflavin deficiency.<sup>(52)</sup>

Both riboflavin and nicotinic acid could be used for the treatment of glossitis. But as for scrotum dermatitis, riboflavin acts much faster than nicotinic acid. Riboflavin is effective in the treatment of perleche but the treatment is rather slow.<sup>(52)</sup>

Etsen Ke-cushing's syndrome is one of the diseases which are caused by endocrine disturbance. During the study of nitrogen, phosphorus and calcium metabolism in Cushing's disease patients, they discovered that these patients need a very high protein diet to maintain their nitrogen balance. The increase of phosphorus and calcium balance always fol-

lows the increase of nitrogen balance. When a patient fed with a high protein diet, the Ca and P in feces will decrease, this may be due to the increase of Ca and P utilization in the body. The phosphorus content in urine is high in patients on low protein diet. If high protein diet is changed to low protein diet, urinary phosphorus will decrease. Because of the fact that the decomposition metabolism is rather strong, the urinary phosphorus increases. The amount of blood phosphorus, calcium and alkaline phosphatase in patients are all within normal limits. The main reason of osteoporosis in this kind patients is that the bony destruction is much faster than bony growth. (53)

The Vitamin C saturation test has proved that Vitamin C deficiency occurs in patient with Cushing's syndrome. The urinary uric acid/urides ratio is 3 times higher than normal individuals. After giving a large amount of Vitamin C, both the uric acid and urides will increase, but the ratio has no change within 24 hours. If a large dose of Vitamin C is given to Cushing's syndrome patients, then the uric acid/urides ratio will increase following the increase of the secretion of Vitamin C. (54)

Ammonium will become abnormal in epileptic patients. During the epileptic attack, the ammonium contents in cerebrospinal fluid is on the average under 0.25 mgm percent. After a grand mal-attack or continuous convulsion, the ammonium may exceed 1 mgm percent, which will gradually decrease to the normal level within half an hour after the attack. (55)

Glutamic acid could be used to cure acute ammonium salt intoxication and hepatic coma. (17-19) In animal experiments, they found the glutamic acid and ATP have no remarkable effect on ammonium intoxication, but arginine has a very good effect.

The fetal hemoglobin of our children is higher than that the same age group in other countries. The fetal hemoglobin of anemic children is even higher. (56)

Tung Tzu-ts'ai and Wang Hsueh-I have determined blood protein-bound-iodine in patients with endemic thyroid enlargement. They discovered that in the endemic area, the P-B-I contents are the same in patients as in normal individuals. But the P-B-I of patients in endemic area is lower than that of normal individuals in non-endemic areas.

#### IV. Test Methods

Since the liberation of the whole country, the test methods in clinical medicine have achieved a great progress. Many new techniques and methods, such as the filter paper electrophoresis, chromatography, micro and ultra-analysis, have been established. Many of the instruments and reagents which were formerly imported from foreign countries are now made by ourselves. This kind of progress is particularly impressive after the great leap-forward in 1958.

1. Protein: There are considerable improvements in the quantitative and qualitative determination of blood and urinary protein. Many researchers(57-61) have simplified the equipment for the determination of blood or urinary protein, and have saved a large amount of reagents. Besides, a comparison of the sensitivity of various urine protein precipitants showed that sulfa-salicylic sodium is most sensitive.(62)

The filter paper electrophoresis method is very simple and easy, and it can be used to analyse various protein compositions by a small amount of sample. This method is generally used in clinical biochemistry laboratories. There are many reports about the improvements, simplification and stain of this method.(3-7,63-65)

2. Enzymes: Kuo Chen-ts'ai(66) has used the sodium permanganate standard solution for the determination of blood peroxylase activity. He also improved this method. Wang Chung and Cheng Chi-chin(67) have studied several conditions for the determination of the activity of glutamic-acetic acid transaminase. The laboratory of Chung-Shan Hospital affiliated with the Shanghai Medical College No 1(68) has established a micro method for amylase determination, and only 10 minutes are needed to complete the whole test.

3. Carbohydrate: As for the tests of urine and blood sugar, Ts'ui Yin(69,70) has used the bitter acid as a reduction reagent in testing the blood sugar and urine sugar. Hsiao Ch'ung-i(71) has used the glycerol-copper reagent for the quantitative determination of urine sugar. Some researchers have established a micro method(72) and other simple methods which can be easily used in rural areas.(73)

4. Non-protein-nitrogen: Tso Shih-shui and others(74) have employed the micro-analytic method (0.2 ml whole blood)

for the determination of N.P.N. Wang Ch'ung and Cheng Chichin(75) have used the sodium sulphuric acid drop method instead of the ordinary digestive method.

5. Urea: Various kind of seeds have been used as the raw material for urea preparations, such as water melon seeds, fresh green bean and horse-bean seeds. They discovered all these seeds contain urease, and the urease in water melon seeds is highly active.(76)

6. Uric acid, muscle acid and muscle anhydride: Chu Hui-tung and others(61) have improved many old methods described in literature, and his methods could save reagents and proved to be more accurate. By Chu's methods, the collection ratio is almost 100 percent.

7. Ammonium: Chiu Chuan-lu and others(18) have improved Conway's method. Ting T'ing and Liu Chao-ch'uan(19) have improved the Tompkins-Kirk tube, and used "3-tube method" for micro analysis of ammonium in blood and cerebrospinal fluid.

8. Cholesterol and phospholipides: Ch'en Pei-en and others(77) have used the anhydride hot acetic acid for the isolation of total cholesterol. This is a simple method, and it minimizes the danger of burning of organic solution. They also improved the method for the determination of total cholesterol and free cholesterol.(78) Li Ch'ien-chai and others(79) have used the filter paper electrophoresis method to divide serum lipid. Then by using micro-analytic method, they determined cholesterol and phospholipides in  $\alpha$  and  $\beta$  lipoprotein.

#### 9. Inorganic salts:

(1) Potassium and sodium: Nitrite cobalt sodium is used directly to precipitate the blood potassium ion, then sulfacyanide is added for staining.(80) Another method for potassium determination is to use the nitrite cobalt sodium to precipitate potassium first, and then the precipitant will stain with double nitrogen.(81) In the area of quantitative determination of blood sodium, uranium-zinc acetic acid is directly added into the blood, and then the precipitant is mixed with protein by centrifuge. This method only needs 0.05 ml of blood and also saves reagents and filter paper.(82)

(2) Calcium and magnesium: EDTA is used for the determination of calcium and magnesium in blood. The researchers have used EDTA directly to measure the total amount of serum calcium and magnesium. Meanwhile, oxamide is used to separate calcium from magnesium, and then the two are determined separately. This method is much simpler than Carr's method used in foreign countries. It saves reagents gives very accurate results. (83)

(3) Protein-bound-iodine: Wang Sun-i (84) has improved the old methods recorded in literature, and increased their sensitivity by 10 times. Only 0.5 ml blood serum is needed for the test. Further improving his method, Wang pointed out the important points in preparing reagents to avoid dirty staining. (85)

(4) Antimony: Ho Cheng-kuan and others (86) have established a speedy chromometer for the determination of the small amount antimony in the blood. This method is very helpful in the observation of the therapeutic effect during drug treatment of schistosomiasis.

Besides, many research units such as the laboratory of Hospital No 1 of the Peking Medical College, (72) the laboratory of Hospital No 1 of the Hunan Medical College (87) and the research unit headed by Wu Chia-ping, (73) have succeeded in the study of microanalytic and ultra-analytic methods for blood or urinary analysis. These methods have many advantages and also increase the efficiency. Besides, as a small amount of blood is needed, the patients suffering and mental anxiety are lessened.

#### V. Biological Products

Since the liberation, the achievements in manufacturing biological products and reagents as mention before will be discussed in other articles. Here we only try to introduce some of these achievements most closely related to biochemistry.

The isolation of bilirubin from urin in jaundice patients is rather convenient and economical (88) because there is no fat and bile salt in urine. A simple method is employed for the preparation of placenta  $\alpha$ -globulin. (89,90) as for the preparation of ACTH from pig's pituitary gland, the product has excellent effect and has no side effects. The preparation procedure is very simple and no refrigera-

tion is needed, and the quantity of products is very large. Satisfactory results have been achieved<sup>(91)</sup> in using soybean instead of casein for the preparation of mixed amino acid. There is a preliminary success in the preparation of fibrino-sponge and fibrino-membrane by using pig's blood. Since its antigenicity still exists, further investigation is needed.<sup>(92)</sup> The preparation of sodium fish liver oil has also been successful and the product meets clinical requirements.<sup>(93)</sup>

From the above, we could see the great achievements in clinical biochemistry since the liberation. From now on, we must raise our work to a higher level both practically and theoretically. On the other hand, we also have to pay more attention to biochemical studies in occupational disease and radiation disease. Due to the development of socialist construction, the study of occupational disease and radiation disease will become a very important subject. As for the prevention of these diseases the traditional Chinese medicine will be very useful, so that we also must pay more attention to the study of the clinical biochemistry of our native medicine. We believe that under the direction of the Communist Party and under the close cooperation of old or young scientist, we will be able to achieve greater successes in the study of clinical biochemistry.

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ACHIEVEMENTS IN THE STUDY OF BIOCHEMISTRY  
AS RELATED TO TRADITIONAL CHINESE  
MEDICINE AND PHARMACOLOGY

Following is a translation of an article entitled "Chung-I Chung-yao Sheng-wu Hsu-hsueh Yen-chiu ti Ch'eng-chiu" (English version above) by the teaching and research group of biochemistry department, Szechuan Medical College, in Ch'ing-chu Chien-kuo Shih-chou-nien I-hsueh K'o-hsueh Ch'eng-chiu Lun-wen-chi (Collected Essays on the Achievements of Medical Science in Celebration of the Tenth Founding Anniversary of the Nation), Vol 1, compiled by the Ministry of Public Health, Peiping, 1959, pp 164-166.

The traditional Chinese medicine and pharmacology is a great valuable treasure accumulated in the course of thousands of years by the Chinese people in their struggle against disease. Before the liberation, biochemistry as related to traditional Chinese medicine and pharmacology was completely an untouched field, because the government completely neglected the development of our traditional medicine and pharmacology. Since the liberation, under the direction of Chinese Communist Party, our biochemical researchers have thoroughly carried out the Party's policy on traditional Chinese medicine. They have realized the importance of heritage and developed our traditional medicine. Many researchers have responded to the Party's call and joined the study of traditional Chinese medicine and pharmacology. They have smashed the superstition and have been working very hard in this field; they have also done a considerable work in the area of clinical biochemistry and its mechanism related to traditional Chinese medicine and pharmacology. The following is an introduction of our achievements in this field:

1. The study of antimicrobial mechanism of traditional Chinese medicine and pharmacology: The members of the teaching and research group of the Szechuan Medical College have conducted a series of studies on the antimicrobial action of huang-lien. (1-4) They discovered that dysentery

bacillus, hemolytic streptococcus and staphylococcus aureus will produce resistance to huang-lien, but, generally, huang-lien is used in combination with other drugs and its effects are good. They have studied the antimicrobial effect and resistance of more than 100 single-taste Chinese drugs and more than 200 Chinese remedies (among which 26 are ancient prescriptions and 181 are new prescriptions). They found that there are about 125 Chinese drugs which have antimicrobial action. Staphylococcus aureus will induce resistance to the single-taste drugs, but some of the old remedies which contain huang-lien will not produce resistance. Such old remedies include "hsieh-hsin-tang," "huang-lien chiai-tu-tanf," and "ko-kun huang-lien-tang." The antimicrobial effects of these old remedies are stronger than the single taste drugs. They concluded that the time honored traditional Chinese medicine has its own scientific value. Recently, on the basis of the theories traditional Chinese medicine, a new type of remedy is introduced for clinical use. Furthermore, they also conducted studies on the mechanism of antimicrobial effect of the traditional Chinese drugs. They have studied the influence of 10 new types of remedies consisting of huang-lien to the respiration of staphylococcus aureus, and their influences to the oxy-glucose, glycerophosphoric acid, lactic acid, acetic acid, succinic acid, citric acid, propyl ketonic acid and corydalin of this bacterium. They also studied the composition of 21 amino acid, 11 vitamins and 11 nucleic acid as related to the action of Chinese drugs. They found that if these drugs could inhibit the growth of staphylococcus aureus, then they could also inhibit the respiration of different bacteria. Meantime, huang-lien has a very strong inhibitory effect on the oxy-decarbonization of propyl-ketonic acid and the dehydrogenation of glucose, lactic acid, succinic acid. Certain Vitamins such as nicotinic acid, Vitamin B6, nucleic acid and nucleoside could resist the inhibitory effect of huang-lien-shu, huang-pe, huang-chin...etc.

Besides, Hsu Chung-lu has established a micro-analysis method of huang-lien in tissue and in body secretions. By this method, he has also studied the absorption, distribution and secretion of huang-lien in the body. He found that huang-lien will distribute in the entire tissue of the body 1 minute after it is injected intravenously; this is very helpful to the study of the effective mechanism of huang-lien.

## 2. The study of the influence of traditional Chinese

drugs to metabolism: Most work was done on tang-kuei, jen-sheng, and kan-t'sao. The members of the biochemistry teaching and research group of the Szechuan Medical College have studied the metabolic influence of tang-kuei, and found that tang-kuei has no remarkable influence on the ribonucleic acid in the liver and uterus tissue of young mice. However, the deoxygenated ribonucleic acid in the uterus increases markedly. Hence, tang-kuei may have a stimulating effect to the growth of uterus.<sup>(5)</sup> They discovered that the consumption of oxygen will increase in mice which had received tang-kuei before the experiment. It also increases the liver tissue's oxidation of glutamic acid and cysteine. This may be related to the Vitamin B<sub>12</sub> content in tang-kuei.<sup>(6)</sup> According to the analytic results of Sun Chih-ling, tang-kuei and several other ordinary used gelatins contain a very large amount of Vitamin B<sub>12</sub>. Besides, Chang Hui-chu and Lin Chih-huan<sup>(7)</sup> have done some analysis of the amino acid contents in ordinary used tonics, such as gelatins obtained from tiger's bone or donkey's skin. They found that the amount of amino acid is the same as the amount of gelatin, except that the amount of lysine and glutamic acid is larger.

In regard to jen-sheng studies, experiments have proved that the jen-sheng solution could increase the sugar fermentation in yeast and tissue section. It is suspected that the release of energy during fermentation may be related to the strong effect of jen-sheng to living body. Sung Cheng-yu and Chien Hsiu-chuan<sup>(8)</sup> have observed that when the rat is stimulated by high or low temperature, then Vitamin C content in adrenal gland will decrease, but if the rat is given jen-sheng before the test, then the Vitamin C content will not show any remarkable change. Wang Cheng-k'ang and Lai Kai-peng<sup>(9)</sup> have used jen-sheng in treating diabetic patients. They found that jen-sheng could inhibit blood sugar in normal dog and diabetic dog, and it could also improve the general physical condition of the diabetic dog, but it could not completely correct the metabolic disturbance of the diabetic dog. The researchers considered that it is very reasonable to use jen-sheng as a supporting treatment for diabetes.

In the study of kan-ts'ao, Lin Shih-hao<sup>(10)</sup> and others have reported 4 cases of Addison's disease treated with kan-ts'ao solution. The patients showed improvements of general condition, increase of serum sodium, and rise of blood pressure after the treatment. A smaller cortison

dose is needed when it is combined with kan-ts'ao. They consider these two drugs have synergistic action. Recently, the clinical medicine teaching and research group of Chang-shun Medical College<sup>(11)</sup> have found that the function of kan-ts'ao is similar to that of adrenal cortical hormone. They used kan-ts'ao for the treatment of Addison's disease, and found an increase of blood sodium and a decrease of potassium in patients.

Sung Cheng-yu and Chen Tseng-hsin<sup>(12)</sup> have done some experiments on Ching-chiu's effect in the treatment of aldehyde arthritis of rats and its influence on the adrenal cortical function. They found ching-chiu could decrease the Vitamin C contents in the adrenal gland of rats.

They have conducted studies on the influence of blood sugar concentration in more than 10 traditional drugs often used by herb practitioners, such as huang-ch'i, ti-huang, pe-shuo, ts'ang-shu and kuo-ko-ken. They found all of these drugs have no effect on the blood sugar except pe-shu and hsiang-fu.<sup>(13-15)</sup> Besides, they also proved that pan-pien-lien, which is effective in treating schistosomiasis, also could increase urinary output and the amount of urinary chloride.<sup>(16,17)</sup>

3. The study of clinical biochemistry of traditional Chinese medicine and pharmacology: Hu Shu-ts'ao and others<sup>(18)</sup> have studied acupuncture for the treatment of lactation deficiency patients. They found that after acupuncture there is an increase of lactation in breast feeding woman. Wang Fu-chou and others<sup>(19,20)</sup> discovered that acupuncture has no effect on the resting blood sugar in rabbit, but it could increase the blood sugar regulating function in sugar-loaded rabbits. Clinical experience has proved that acupuncture could regulate the gastric secretion in patients with chronic gastritis and gastric ulcer. This is because acupuncture can decrease gastric acid or pepsinase when they are insufficient.<sup>(21)</sup> Besides, they also did some study on the acupuncture in relation to adrenal cortical function.<sup>(22)</sup>

The basic internal medicine teaching and research group of the Sian Medical College<sup>(23)</sup> have reported 2 cases treated with Vitamin B<sub>12</sub>, the injection of which was guided by the location of acupuncture. A very satisfactory result was obtained, and only 0.015 mgm of Vitamin B<sub>12</sub> was used, while the ordinary therapeutic dose is 30-60 mgm intramus-

cularly.

The Sian Medical College(24) used willow branch to unite a fractured bone in its clinic. It was discovered that after the operation, the activity of acid and alkaline phosphatase increases 10-40 times as compared with normal individuals. They considered that willow branch contains methyl phosphoric acid which could stimulate the growth of the plant and may also increase the activity of phosphatase.

The above are the achievements in the study of biochemistry as related to traditional Chinese medicine and pharmacology after the liberation. This is only a good beginning in field of Chinese medicine research, and still far from being able to meet people's needs. The traditional Chinese medicine has thousands of years of history, and it is very important in the treatment of disease. Our biochemical workers must co-operate with other workers specialized in clinical medicine, physiology and pathological physiology, under the Party's direction. They should all together and try to clarify the therapeutic mechanism of traditional Chinese medicine and pharmacology. This will be helpful not only for the development of our traditional medicine, but also for the development medical science in the whole world.

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